NRC FORM 374
(7-94)

U.S. NUCLEAR REGULATORY COMMISSION

MATERIALS LICENSE

Amendment No. 04

Pursuant to the Atomic Energy Act of 1954, as amended, the Energy Reorganization Act of 1974 (Public Law 93-438), and Title 10, Code of Federal Regulations, Chapter I, Parts 30, 31, 32, 33, 34, 35, 36, 39, 40, and 70, and in reliance on statements and representations heretofore made by the licensee, a license is hereby issued authorizing the licensee to receive, acquire, possess, and transfer byproduct, source, and special nuclear material designated below; to use such material for the purpose(s) and at the place(s) designated below; to deliver or transfer such material to persons authorized to receive it in accordance with the regulations of the applicable Part(s). This license shall be deemed to contain the conditions specified in Section 183 of the Atomic Energy Act of 1954, as amended, and is subject to all applicable rules, regulations, and orders of the Nuclear Regulatory Commission now or hereafter in effect and to any conditions specified below.

397896

Licensee	
1. Novagen, Inc. 2. 601 Science Drive Madison, WI 53711	In accordance with application dated November 28, 1996 3. License Number 48-26067-01 is renewed in its entirety to read as follows: 4. Expiration Date December 31, 2001 5. Docket or Reference No. 030-31338

6. Byproduct, Source, and/or Special Nuclear Material	7. Chemical and/or Physical Form	8. Maximum Amount that Licensee May Possess at Any One Time Under This License
A. Carbon-14	A. Any	A. 5 millicuries
B. Hydrogen-3	B. Any	B. 5 millicuries
C. Phosphorus-32	C. Any	C. 25 millicuries
D. Phosphorus-33	D. Any	D. 25 millicuries
E. Sulfur-35	E. Any	E. 25 millicuries

9. Authorized Use:

A. through E. To be used for research and development as defined in 10 CFR Part 30, Section 30.4.

CONDITIONS

10. Licensed material shall be used only at the licensee's facilities located at 597 and 601 Science Drive, Madison, Wisconsin.

11. Licensed material shall be used by, or under the supervision of, Robert C. Mierendorf, Ph.D., Barbara Morris, Corrine Fetherston, Robert Novy, or Thomas VanOosbree, Ph.D.

12. The Radiation Safety Officer for this license is Robert C. Mierendorf, Ph.D.

13. Survey instruments may be calibrated by the University of Wisconsin-Madison (NRC License No. 48-00983-18) or by any individual or firm authorized to provide such services under an NRC or Agreement State license, on an annual basis.

ML30

190038

COPY 4

MATERIALS LICENSE
SUPPLEMENTARY SHEET

License Number
48-26067-01

Docket or Reference Number
030-31338

Amendment No. 04

14. The licensee is authorized to hold radioactive material with a physical half-life of less than 90 days for decay-in-storage before disposal in ordinary trash provided:

A. Radioactive waste to be disposed of in this manner shall be held for decay a minimum of 10 half-lives.

B. Before disposal as ordinary trash, byproduct material shall be surveyed at the container surface with the appropriate survey meter set on its most sensitive scale and with no interposed shielding to determine that its radioactivity cannot be distinguished from background. All radiation labels shall be removed or obliterated.

C. A record of each disposal permitted under this License Condition shall be retained for three years. The record must include the date of disposal, the date on which the byproduct material was placed in storage, the radionuclides disposed, the survey instrument used, the background dose rate, the dose rate measured at the surface of each waste container, and the name of the individual who performed the disposal.

14. Except as specifically provided otherwise in this license, the licensee shall conduct its program in accordance with the statements, representations, and procedures contained in the documents, including any enclosures, listed below. The U.S. Nuclear Regulatory Commission's regulations shall govern unless the statements, representations, and procedures in the licensee's application and correspondence are more restrictive than the regulations.

A. Application dated November 28, 1994; and

B. Letter dated September 20, 1996.

FOR THE U.S. NUCLEAR REGULATORY COMMISSION

Date December 6, 1996

By Patricia J. Pelke
Nuclear Materials Licensing Branch, Region III

COPY

MATERIALS LICENSE

Amendment No. 04

Pursuant to the Atomic Energy Act of 1954, as amended, the Energy Reorganization Act of 1974 (Public Law 93-438), and Title 10, Code of Federal Regulations, Chapter I, Parts 30, 31, 32, 33, 34, 35, 36, 39, 40, and 70, and in reliance on statements and representations heretofore made by the licensee, a license is hereby issued authorizing the licensee to receive, acquire, possess, and transfer byproduct, source, and special nuclear material designated below; to use such material for the purpose(s) and at the place(s) designated below; to deliver or transfer such material to persons authorized to receive it in accordance with the regulations of the applicable Part(s). This license shall be deemed to contain the conditions specified in Section 183 of the Atomic Energy Act of 1954, as amended, and is subject to all applicable rules, regulations, and orders of the Nuclear Regulatory Commission now or hereafter in effect and to any conditions specified below.

397896

Licensee	
1. Novagen, Inc.	In accordance with application dated November 28, 1996
2. 601 Science Drive Madison, WI 53711	3. License Number 48-26067-01 is renewed in its entirety to read as follows:
	4. Expiration Date December 31, 2001
	5. Docket or Reference No. 030-31338

6. Byproduct, Source, and/or Special Nuclear Material	7. Chemical and/or Physical Form	8. Maximum Amount that Licensee May Possess at Any One Time Under This License
A. Carbon-14	A. Any	A. 5 millicuries
B. Hydrogen-3	B. Any	B. 5 millicuries
C. Phosphorus-32	C. Any	C. 25 millicuries
D. Phosphorus-33	D. Any	D. 25 millicuries
E. Sulfur-35	E. Any	E. 25 millicuries

9. Authorized Use:

A. through E. To be used for research and development as defined in 10 CFR Part 30, Section 30.4.

CONDITIONS

10. Licensed material shall be used only at the licensee's facilities located at 597 and 601 Science Drive, Madison, Wisconsin.

11. Licensed material shall be used by, or under the supervision of, Robert C. Mierendorf, Ph.D., Barbara Morris, Corrine Fetherston, Robert Novy, or Thomas VanOosbree, Ph.D.

12. The Radiation Safety Officer for this license is Robert C. Mierendorf, Ph.D.

13. Survey instruments may be calibrated by the University of Wisconsin-Madison (NRC License No. 48-00983-18) or by any individual or firm authorized to provide such services under an NRC or Agreement State license, on an annual basis.

MATERIALS LICENSE
SUPPLEMENTARY SHEET

License Number
48-26067-01

Docket or Reference Number
030-31338

Amendment No. 04

14. The licensee is authorized to hold radioactive material with a physical half-life of less than 90 days for decay-in-storage before disposal in ordinary trash provided:

A. Radioactive waste to be disposed of in this manner shall be held for decay a minimum of 10 half-lives.

B. Before disposal as ordinary trash, byproduct material shall be surveyed at the container surface with the appropriate survey meter set on its most sensitive scale and with no interposed shielding to determine that its radioactivity cannot be distinguished from background. All radiation labels shall be removed or obliterated.

C. A record of each disposal permitted under this License Condition shall be retained for three years. The record must include the date of disposal, the date on which the byproduct material was placed in storage, the radionuclides disposed, the survey instrument used, the background dose rate, the dose rate measured at the surface of each waste container, and the name of the individual who performed the disposal.

14. Except as specifically provided otherwise in this license, the licensee shall conduct its program in accordance with the statements, representations, and procedures contained in the documents, including any enclosures, listed below. The U.S. Nuclear Regulatory Commission's regulations shall govern unless the statements, representations, and procedures in the licensee's application and correspondence are more restrictive than the regulations.

A. Application dated November 28, 1994; and

B. Letter dated September 20, 1996.

FOR THE U.S. NUCLEAR REGULATORY COMMISSION

Date December 6, 1996

By Patricia J. Pelke
Nuclear Materials Licensing Branch, Region III

COPY

BETWEEN:

LICENSE FEE MANAGEMENT BRANCH, ARM
AND
REGIONAL LICENSING SECTIONS

(FOR LFMS USE)
INFORMATION FROM LTS

PROGRAM CODE: 03620
STATUS CODE: 2
FEE CATEGORY: 3M
EXP. DATE: 19941231
FEE COMMENTS:
DECOM FIN ASSUR REQDT R

LICENSE FEE TRANSMITTAL

A. REGION

1. APPLICATION ATTACHED
APPLICANT/LICENSEE: NOVAGEN
RECEIVED DATE: 941129
DOCKET NO: 3031338
CONTROL NO.: 397896
LICENSE NO.: 48-26067-01
ACTION TYPE: RENEWAL

2. FEE ATTACHED
AMOUNT: 1500.00
CHECK NO.: 401237

3. COMMENTS

SIGNED D. Hersey
DATE 12-5-94

B. LICENSE FEE MANAGEMENT BRANCH (CHECK WHEN MILESTONE 03 IS ENTERED ✓)

1. FEE CATEGORY AND AMOUNT: 3M $1500.00

2. CORRECT FEE PAID, APPLICATION MAY BE PROCESSED FOR:
AMENDMENT
RENEWAL ✓
LICENSE

3. OTHER

SIGNED JC
DATE 12/7/94

RECEIVED
DEC 12
REGION III

NRC FORM 313 U. S. NUCLEAR REGULATORY COMMISSION

(6-93)
10 CFR 30, 32, 33
34, 35, 36, 39 and 40

APPLICATION FOR MATERIAL LICENSE

APPROVED BY OMB: NO. 3150-0120
EXPIRES 6-30-96

ESTIMATED BURDEN PER RESPONSE TO COMPLY WITH THIS INFORMATION COLLECTION REQUEST: 9 HOURS. SUBMITTAL OF THE APPLICATION IS NECESSARY TO DETERMINE THAT THE APPLICANT IS QUALIFIED AND THAT ADEQUATE PROCEDURES EXIST TO PROTECT THE PUBLIC HEALTH AND SAFETY. FORWARD COMMENTS REGARDING BURDEN ESTIMATE TO THE INFORMATION AND RECORDS MANAGEMENT BRANCH (MNBB 7714), U.S. NUCLEAR REGULATORY COMMISSION, WASHINGTON, DC 20555-0001, AND TO THE PAPERWORK REDUCTION PROJECT (3150-0120), OFFICE OF MANAGEMENT AND BUDGET, WASHINGTON, DC 20503.

INSTRUCTIONS: SEE THE APPROPRIATE LICENSE APPLICATION GUIDE FOR DETAILED INSTRUCTIONS FOR COMPLETING APPLICATION. SEND TWO COPIES OF THE ENTIRE COMPLETED APPLICATION TO THE NRC OFFICE SPECIFIED BELOW.

APPLICATION FOR DISTRIBUTION OF EXEMPT PRODUCTS FILE APPLICATIONS WITH:

DIVISION OF INDUSTRIAL AND MEDICAL NUCLEAR SAFETY
OFFICE OF NUCLEAR MATERIALS SAFETY AND SAFEGUARDS
U.S. NUCLEAR REGULATORY COMMISSION
WASHINGTON, DC 20555-0001

ALL OTHER PERSONS FILE APPLICATIONS AS FOLLOWS:

IF YOU ARE LOCATED IN:

CONNECTICUT, DELAWARE, DISTRICT OF COLUMBIA, MAINE, MARYLAND, MASSACHUSETTS, NEW HAMPSHIRE, NEW JERSEY, NEW YORK, PENNSYLVANIA, RHODE ISLAND, OR VERMONT, SEND APPLICATIONS TO:

LICENSING ASSISTANT SECTION
NUCLEAR MATERIALS SAFETY BRANCH
U.S. NUCLEAR REGULATORY COMMISSION, REGION I
475 ALLENDALE ROAD
KING OF PRUSSIA, PA 19406-1415

ALABAMA, FLORIDA, GEORGIA, KENTUCKY, MISSISSIPPI, NORTH CAROLINA, PUERTO RICO, SOUTH CAROLINA, TENNESSEE, VIRGINIA, VIRGIN ISLANDS, OR WEST VIRGINIA, SEND APPLICATIONS TO:

NUCLEAR MATERIALS LICENSING SECTION
U.S. NUCLEAR REGULATORY COMMISSION, REGION II
101 MARIETTA STREET, NW, SUITE 2900
ATLANTA, GA 30323-0199

IF YOU ARE LOCATED IN:

ILLINOIS, INDIANA, IOWA, MICHIGAN, MINNESOTA, MISSOURI, OHIO, OR WISCONSIN, SEND APPLICATIONS TO:

MATERIALS LICENSING SECTION
U.S. NUCLEAR REGULATORY COMMISSION, REGION III
801 WARRENVILLE RD.
LISLE, IL 60532-4351

ARKANSAS, COLORADO, IDAHO, KANSAS, LOUISIANA, MONTANA, NEBRASKA, NEW MEXICO, NORTH DAKOTA, OKLAHOMA, SOUTH DAKOTA, TEXAS, UTAH, OR WYOMING, SEND APPLICATIONS TO:

NUCLEAR MATERIALS LICENSING SECTION
U.S. NUCLEAR REGULATORY COMMISSION, REGION IV
611 RYAN PLAZA DRIVE, SUITE 400
ARLINGTON, TX 76011-8064

ALASKA, ARIZONA, CALIFORNIA, HAWAII, NEVADA, OREGON, WASHINGTON, AND U.S. TERRITORIES AND POSSESSIONS IN THE PACIFIC, SEND APPLICATIONS TO:

RADIOACTIVE MATERIALS SAFETY BRANCH
U.S. NUCLEAR REGULATORY COMMISSION, REGION V
1450 MARIA LANE
WALNUT CREEK, CA 94596-5368

PERSONS LOCATED IN AGREEMENT STATES SEND APPLICATIONS TO THE U.S. NUCLEAR REGULATORY COMMISSION ONLY IF THEY WISH TO POSSESS AND USE LICENSED MATERIAL IN STATES SUBJECT TO U.S.NUCLEAR REGULATORY COMMISSION JURISDICTIONS.

1. THIS IS AN APPLICATION FOR *(Check appropriate item)*

- [ ] A. NEW LICENSE
- [ ] B. AMENDMENT TO LICENSE NUMBER _____
- [X] C. RENEWAL OF LICENSE NUMBER 48-26067-01

2. NAME AND MAILING ADDRESS OF APPLICANT *(Include Zip code)*

Novagen, Inc.
597 Science Drive
Madison, WI 53711

3. ADDRESS(ES) WHERE LICENSED MATERIAL WILL BE USED OR POSSESSED

597 and 595 Science Drive
Madison, WI 53711

4. NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

Robert Mierendorf

TELEPHONE NUMBER
608-238-6110

SUBMIT ITEMS 5 THROUGH 11 ON 8-1/2 X 11" PAPER. THE TYPE AND SCOPE OF INFORMATION TO BE PROVIDED IS DESCRIBED IN THE LICENSE APPLICATION GUIDE.

5.	RADIOACTIVE MATERIAL a. Element and mass number, b. chemical and/or physical form, and c. maximum amount which will be possessed at any one time.	6.	PURPOSE(S) FOR WHICH LICENSED MATERIAL WILL BE USED
7.	INDIVIDUAL(S) RESPONSIBLE FOR RADIATION SAFETY PROGRAM AND THEIR TRAINING EXPERIENCE	8.	TRAINING FOR INDIVIDUALS WORKING IN OR FREQUENTING RESTRICTED AREAS
9.	FACILITIES AND EQUIPMENT	10.	RADIATION SAFETY PROGRAM
11.	WASTE MANAGEMENT	12.	LICENSE FEES *(See 10 CFR 170 and Section 170.31)* FEE CATEGORY 3M AMOUNT ENCLOSED $ 1500

13. CERTIFICATION *(Must be completed by applicant)* THE APPLICANT UNDERSTANDS THAT ALL STATEMENTS AND REPRESENTATIONS MADE IN THIS APPLICATION ARE BINDING UPON THE APPLICANT.

THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATION ON BEHALF OF THE APPLICANT, NAMED IN ITEM 2, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PARTS 30, 32, 33, 34, 35, 36, 39 AND 40, AND THAT ALL INFORMATION CONTAINED HEREIN IS TRUE AND CORRECT TO THE BEST OF THEIR KNOWLEDGE AND BELIEF.

WARNING: 18 U.S.C. SECTION 1001 ACT OF JUNE 25, 1948 62 STAT. 749 MAKES IT A CRIMINAL OFFENSE TO MAKE A WILLFULLY FALSE STATEMENT OR REPRESENTATION TO ANY DEPARTMENT OR AGENCY OF THE UNITED STATES AS TO ANY MATTER WITHIN ITS JURISDICTION.

CERTIFYING OFFICER – TYPED/PRINTED NAME AND TITLE	SIGNATURE	DATE
Robert Mierendorf, Vice President	[signature] RECEIVED	11/28/94

FOR NRC USE ONLY

TYPE OF FEE	FEE LOG	FEE CATEGORY	AMOUNT RECEIVED	CHECK NUMBER	COMMENTS
Renewal	Dec 2 III	3M	$1500.00	404234	NOV 29 1994
APPROVED BY			DATE 12/7/94		REGION III 397896

Novagen

Novagen, Inc. 597 Science Dr. Madison, WI 53711 Telephone (608) 238-6110 Fax (608) 238-1388

Materials Licensing Section
U.S. Nuclear Regulatory Commission, Region III
801 Warrenville Road
Lisle, IL 60532-4351

November 28, 1994

Dear Reviewer,

We are submitting a complete up-to-date renewal application for our NRC license (# 48-26067-01). We have made no references to previous submittals. I have indicated below the most significant changes which have been written for the renewed license:

1. Addition of Carbon-14 and Phosphorous-33 as radioactive materials for possession and use. Possession limits for Carbon-14 will be 5mCi and Phosphorous-33 will be 25mCi. Increasing the possesion limit to 25mCi for Phosphorous-32. See section 5.

2. Deletion of Richard L. Garber and Chris A. Percy as authorized users. Addition of Thomas R. Van Oosbree as authorized user. See section 7.

N/A never occupied per VH 2LD. 9/20/94

3. Addition of a new laboratory at 595 Science Drive, Madison, WI, which is next to our current facility at 597 Science Drive. The new laboratory is separated from the rest of the building by walls and doors. See section 9 for a description of the new laboratory. Also included with the renewal is the wipe test map for the new laboratory.

4. Addition of decay-in-storage for Sulfur-35. We request to be authorized to hold radioactive material with a physical half-life of less than 90 days (specifically Sulfur-35) for decay-in-storage before disposal in ordinary trash. See section 11. The Sulfur -35 waiting to be placed in decay-in-storage is 1.8 cubic feet with an activity of 0.58mCi. We will not need any additional permits or approvals for storage.

Enclosed is a check for $1500 to cover the costs of the license renewal.

Please call me if I can be of further assistance.

Sincerely,

Robert C. Mierendorf
Vice President and General Manager

5. **Radioactive material**

   a. Element and mass number:
   ^{14}C, ^{3}H, ^{32}P, ^{33}P, ^{35}S

   b. Chemical and/or physical form:
   any

   c. Maximum amount possessed at any one time:
   5mCi of ^{14}C; 5mCi of ^{3}H; 25mCi of ^{32}P; 25mCi of ^{33}P; 25mCi of ^{35}S

6. **Purpose(s) for which licensed material will be used:**
Research, development and quality control of molecular biological reagents. See attached protocols.

7. **Individual(s) responsible for radiation safety program and their training and experience:**

Robert C. Mierendorf, Ph. D.:
6/89-present: Vice President, General Manager, and Radiation Protection Officer, Novagen, Inc., Madison , WI.

4/84-5/89: Senior Scientist, Director of Research and Development, Promega Corporation (2800 Woods Hollow Road, Madison, WI 53711). Used up to 10 mCi ^{32}P labeled inorganic phosphate, ATP, CTP, dATP and dCTP, and up to 1 mCi ^{3}H CTP and dCTP at a time for development and quality control of molecular biology reagents. Trained personnel in the handling of radioisotopes; supervised record keeping, isotope disposal, and wipe testing for department.

8/81-3/84: Postdoctoral trainee and project associate, Department of Bacteriology, University of Wisconsin, Madison, WI. Used up to 5 mCi ^{35}S methionine and sulfate, and up to 1 mCi ^{32}P phosphate at a time for research in protein synthesis and processing.

9/74-7/81: Graduate trainee and postdoctoral trainee, Department of Oncology, University of Wisconsin, Madison, WI. Used up to 2 mCi ^{3}H-dTTP, -dCTP, -UTP, -CTP, -uridine,-dexamethasone, and -estradiol, up to 250 µCi ^{14}C-biotin and formaldehyde, and up to 1 mCi ^{32}P-dCTP and -UTP at a time for research in cell and molecular biology of steroid hormone action. Attended semester course on the use and handling of radioisotopes. Passed radiation safety exam.

Barbara B. Morris:
6/89-present: Senior Scientist, Technical Services, Novagen, Inc., Madison, WI.

9/86-5/89: Scientist in Production and Research and Development Departments at Promega Corp. (2800 Woods Hollow Road, Madison, WI, 53711). Used up to 250 µCi ^{32}P-dCTP at a time for development and quality control of molecular biology reagents. Was responsible for keeping written records of isotope receipt, use, and disposal for entire company. Assisted with weekly wipe tests.

9/81-8/86: Research Assistant at University of Wisconsin, Madison, departments of Genetics and Plant Pathology. Used up to 100 μCi ^{32}P-dCTP to label DNA for hybridizations. Instructed students in a laboratory class which included lessons on the handling of radioisotopes. Passed radiation safety exam.

8/87-12/80: Graduate student at University of MD, College Park, Dept. Botany. Took courses in laboratory techniques and safety. Topics covered included Radiation Theory and Detection. Passed radiation safety exam.

10/76- 8/78: Biological Technician at the USDA Soilborne Diseases Lab, Beltsville, MD . Used up to 250 μCi ^{14}C-carboxin to trace the translocation of systemic fungicides in cotton and soybeans.

Corrine Fetherston:
6/89-present: Senior Scientist, Manufacturing and Marketing, Novagen, Inc., Madison, WI.

5/88- 5/89: Scientist in Research and Development at Promega Corp. (2800 Woods Hollow Rd., Madison, WI, 53711). Used up to 250 μCi ^{32}P-dCTP and -ATP at a time to label DNA for hybridizations. Responsible for receiving and distribution of radioisotopes for the entire company.

9/85- 3/88: Research Specialist at the University of Illinois at Chicago, Dept. Opthalmology. Used up to 100 μCi ^{32}P-dCTP for labeling DNA probes and 300 μCi ^{35}S-UTP at a time for making RNA probes. Completed a required course in radiation safety given by the University Radiation Safety Dept.

4/84- 6/86: Research Technician at Agrigenetics (5649 E. Buckeye Rd., Madison, WI, 53716). Used up to 40 μCi ^{32}P-dCTP to make DNA probes and 100 μCi ^{125}I-protein A for Western blotting. Passed required radiation safety exam. Assisted with weekly wipe tests.

9/81- 10/83: Research Assistant at University of Wisconsin, Madison, Dept. Bacteriology. Used up to 50 μCi ^{14}C-glucose for metabolism studies. Passed radiation safety exam.

Robert E. Novy
6/91-present: Senior Scientist, Research and Development, Novagen, Inc., Madison, WI.

8/85- 5/91: Research assistant and graduate student at University of Iowa, Iowa City, IA Used ^{125}I- labeled antibodies for Western blots and immunoscreening. ^{32}P used for sequencing, end labeling probes and uniform labeled probes. Used up to 75μCi per assay for sequencing. Used up to 75μCi ^{35}S per assay for sequencing.

2/84- 7/85: Technician at Mayo Clinic, Rochester, MN.
Successfully completed course in radiation information and protection. Performed ^{125}I- labeling of antibodies, using 500μCi ^{125}I per labeling reaction. Used labeled antibodies for radioimmunoassays.

Thomas R. Van Oosbree, Ph. D:
9/92- present: Senior Scientist, Protein Biochemistry, Novagen, Inc., Madison, WI.

6/84-8/92: Senior Scientist, Protein Biochemistry Group Leader, Promega Corporation (2800 Woods Hollow Road, Madison, WI 53711). Used up to 1mCi ^{32}P labeled inorganic phosphate, ATP, CTP, and dATP, up to 1mCi ^{3}H CTP and ATP, up to 1mCi ^{35}S methionine at a time for development and quality control of molecular biology reagents. Trained personnel in the handling of radioisotopes; supervised record keeping, isotope disposal, and wipe testing for company. Passed radiation safety exam and attended seminar on use and handling of radioisotopes.

9/83-5/84: Postdoctoral Fellow, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI. Used up to 1mCi ^{125}I for labeling proteins.

7/77-8/83: Graduate student, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI. Used up to 1mCi ^{3}H CTP, dTTP, dexamethasone, and estradiol, up to 1mCi ^{32}P dCTP at a time for research in cell and molecular biology of steroid hormone action. Attended semester course on the use and handling of radioisotopes. Passed radiation safety exam.

9/75- 6/77: Research assistant, Department of Zoology, University of Iowa, Iowa City, Iowa. Used up to 1mCi of ^{3}H and ^{14}C amino acids for *in vivo* and *in vitro* protein synthesis studies. Passed radiation safety exam.

Radiation Protection Officer
Dr. Robert Mierendorf shall be Novagen's Radiation Protection Officer. His duties and responsibilities shall include:

a. To ensure that the use of radioactive material is by or under the direct supervision of individuals specifically listed on the license.

b. To ensure that all users (where appropriate) wear personal monitoring equipment when using radioactive materials.

c. To ensure that radioactive materials are properly secured against unauthorized removal at all times when not in use.

d. To perform routine inspections of all laboratories using or storing radioactive materials and/or leak tests of sealed sources.

e. To ensure that the terms and conditions of the license are met, and that all required records are maintained.

f. To immediately halt any activity judged to be a threat to health, safety, the environment or a violation of the conditions of the license or the regulations.

8. Training for individuals working in or frequenting restricted areas:

The documents specified in 10 CFR Part 19, Section 19.11 shall be posted at the entrance to the restricted area. All individuals working in or frequenting any portion of the restricted area shall be instructed according to 10 CFR Part 19, Section 19.12. Specific training for all protocols will be provided to laboratory personnel by the Radiation Protection Officer or his designee.

All personnel working in or frequenting restricted areas will receive a copy of the handbook, "Radiation Safety for Radiation Workers", developed by the University of Wisconsin Safety Department. This document was written for individuals who anticipate working with sources of ionizing radiation or small amounts of radioactive materials in the fields of medicine and research. It provides basic instruction in radiation and the health protection problems associated with exposure to radioactive materials, procedures and equipment used to monitor and minimize radiation exposure, and detailed safety procedures for the transfer, storage and handling of radioactive materials in compliance with NRC regulations. The scope of radionuclides described in the handbook includes those to be used at Novagen. Successful completion of a Radiation Safety Exam will be required by all personnel before being allowed to work with radioisotopes. Refresher exams will be administered every three years to radioisotope users.

In addition, each person will receive written general instructions to be followed while working with radioactive materials at Novagen. A copy of this document is attached.

All employees will observe the prohibitions against eating, drinking, smoking, and applying cosmetics in the labs in which radioisotopes are used. All areas where radioisotopes are used and stored will be clearly marked and all personnel will be instructed to minimize their exposure by reducing the time spent in the area, and increasing the distance or shielding between themselves and radioactive materials.

Support personnel (such as clerical and janitorial personnel) will be informed about radiation hazards and appropriate precautions when they first start working at Novagen and once a year thereafter. A one-page document will be distributed to these personnel and will include:

1. A general explanation that isotopes are being used and that proper precautions are necessary.
2. A description of the signs and markings used to designate restricted areas.
3. Precautionary measures to be used in restricted areas, such as no smoking, eating, drinking, or applying cosmetics (cleaning personnel will be given the additional instruction that they are not to touch marked areas or waste containers).

This document will also be discussed with appropriate supervisory personnel of contract cleaning services.

9. Facilities and equipment:

(For facilities, see attached building floor plans).
Areas which will be designated for use with isotopes:
a. The two large laboratories (Labs 1 and 2 on the map) at 597 Science Drive with four small work areas for specific functions: isotope lab, tissue culture room, microbiology lab and dark room.
b. The laboratory at 595 Science Drive with one small work area for a specific function: microbiology/tissue culture room.

Equipment: Manipulations of ^{32}P and ^{33}P radioisotopes will be performed behind 3/8 inch acrylic shields and workers will wear protective attire, such as lab coats and disposable gloves. An appropriately calibrated G-M survey meter (Research Products International Model 900-E or GM2; sensitivity to a 1 mR/hr ^{60}Co gamma radiation field = 29cps, or equivalent) will be used to monitor work areas before and after isotope use. The instrument will be calibrated by the University of Wisconsin-Madison, Radiation Calibration Laboratory, 1300 Univeristy Avenue, Room 1530, Madison, WI 53706 (NRC license number 48-00361-18).

The following equipment will be dedicated to use with radioisotopes: -20°C freezer, -70°C freezer, 4°C cold box, microcentrifuge, adjustable micropipets (which use disposable tips), vortex mixer, sidearm filtering flask, gel dryer, gel electrophoresis units, heating blocks. A scintillation counter will be used for experiments and to determine wipe test survey results.

10. Radiation safety program:

Standard laboratory procedures will be followed in the restricted lab areas, in addition to procedures described in the attached document, "General Instructions for Radioisotope Users". All personnel will wear lab coats and disposable gloves when working with any radioisotopes. The lab coats and gloves will be removed and left in a designated area when workers leave the lab. Personal dosimeter badges will be worn by workers using ^{32}P and ^{33}P. All microcentrifugation of radiolabeled material will be performed in screw-top microcentrifuge tubes with rubber O-rings to minimize aerosol formation. Precautionary procedures will be in accordance with 10 CFR Part 20, Sections 20.201-20.207

Special safety precautions for the use of >1 mCi ^{32}P at one time:

a. Mandatory radiation survey and wipe test after each use.
b. Use of finger type extremity monitors.
c. Use of a dry run prior to the performance of unfamiliar procedures.
d. The Radiation Protection Officer will be present during new procedures.

Wipe tests and their frequency: Monthly wipe tests will be performed on lab bench tops, the hood, lab equipment, door handles, and other areas of possible contamination (see enclosed wipe test maps). An area 100 cm^2 will be wiped with a 3 cm x 3 cm square of tissue moistened with 70% ethanol. The tissue will be placed in a scintillation vial to which scintillation cocktail will be added. Samples will be counted in a scintillation counter. Any area more than 1000 cpm will be considered contaminated and will be cleaned with decontaminanting detergent and water until wipe tests show levels of 1000 cpm or less. The internal surfaces of certain pieces of equipment, such as the microcentrifuge, will likely become contaminated. Contamination inside of this equipment will be permitted if exposures on the outer surfaces are less than 2 mR/hr. For low energy beta emitters, contamination inside the equipment will not exceed 22,000 dpm/cm^2.

Ordering and receiving radioactive materials: Authorized users who wish to order isotopes will submit their requests to Novagen's purchasing agent. The purchasing agent will consult with the radiation protection officer or the isotope log book to ensure that receipt of the requested material will not exceed possession limits. If the request is within the possession limits, the purchasing agent will complete a purchase order. If the request exceeds the possession limit, the purchasing agent will inform the user that the material cannot be ordered.

All shipments of radioactive material are delivered to Novagen's receiving area. All Novagen personnel will be instructed to notify the Radiation Protection Officer or another authorized user immediately upon receipt of radioactive material. The Radiation Protection Officer or another authorized user will inspect the packages for leakage, damage, or contamination and place the package in a designated storage area in the isotope lab within three hours after receipt. The person inspecting incoming packages will wear gloves and monitor the packages before opening (except for packages containing material exempted in 10 CFR Part 20, Section 20.205). The packing material will be monitored after the package is opened. If contamination above 22,000 dpm/100 cm^2 is detected on the outside of the package, Novagen will immediately notify the carrier and the NRC as specified in 10 CFR Part 20, Section 20.205

11. Waste management:

Waste disposal will be in accordance with 10 CFR Part 20, Sections 20.301-20.311. ^{32}P and ^{33}P waste, both with half-lives less than 65 days, will be stored in shielded containers for 10 half-lives, then disposed with other nonradioactive ordinary trash. ^{35}S, with a half-life of 87.4 days, will also be kept for decay over 10 half-lives in an appropriately labeled container. See below for procedures used for decay in storage of ^{35}S waste.

Other procedures for waste management are detailed in the attached document "General Instructions for Radioisotope Users".

^{35}S Decay In Storage:

The following procedures will be used for decay-in-storage of ^{35}S before disposal in ordinary trash:

a. Geiger-Mueller Monitors (Models 900-E and GM2, Research Products International, Mount Prospect, Illinois) will be used for surveying ^{35}S waste for decay-in-storage. The minimum detectable activity for the instruments is $2 \times 10^{-3}\mu Ci$ of ^{35}S. The final survey will be performed in a low radiation background area with all shielding removed.

b. The waste will be segregated to ensure that ^{35}S decay-in-storage is separately identified from interim storage wastes. The waste containers will be labeled as "^{35}S Decay-In-Storage" along with the yellow label which has a purple or magenta three bladed radiation symbol. The waste will be stored in a 3/8" thick acrylic container with a hinged lid. The lid will be locked at all times except to perform duties as listed below. The volume of the acrylic container will be approximately 4 cubic feet. Up to ten plastic bags of approximately 0.4 cubic feet each will be placed in the acrylic waste container. Each bag will be labeled with an identification number and the date placed in storage. Records for date placed in storage, date removed, and survey results will be kept for each plastic bag kept in the waste container. The ^{35}S waste will be inspected and surveyed monthly. Record sheets for ^{35}S decay-in-storage are enclosed. The maximum volume of ^{35}S for decay-in-storage will be 4 cubic feet with a maximum activity of 20mCi. The physical form of the waste will be solid. There will be no waste processing, such as volume reduction, solidification or other treatment.

c. The ^{35}S waste will be held for a minimum of 10 half-lives (30 months).

d. The decayed ^{35}S waste will not be disposed of as ordinary trash unless the final survey results are indistinguishable from background radiation, using the survey procedures discussed in Paragraph a, above, and radiation labels are obliterated or removed. Before disposal, the radioactive material in the container at the container surface will be monitored to determine that its radioactivity cannot be distinguished from background radiation levels. A record of each disposal of ^{35}S waste will be retained for 3 years. The record will include the date of the disposal, the date on which the ^{35}S waste was placed in storage, the radionuclide disposed of, the survey instrument used, the background dose rate, the dose rate measured at the surface of each waste container, and the name of the individual who performed the disposal.

General Instructions for the Use of Radioisotopes:

a. *Control Procedures: obtaining permission, limitations on quantities/experiment:*

1. All personnel must read the University of Wisconsin Manual, "Radiation Safety for Radiation Workers", and successfully complete the radiation safety exam in order to use radioactive materials at Novagen.

2. Novagen's NRC license covers the use of ^{14}C, ^{3}H, ^{35}S, ^{32}P, and ^{33}P. The company may possess a total of 5mCi of ^{14}C, 5mCi of ^{3}H, 25mCi of ^{32}P, 25mCi of ^{33}P, and 25mCi of ^{35}S at any one time. Consequently, you must check on current levels with the Radiation Protection Officer before ordering isotope. Up to 1mCi of ^{14}C or ^{3}H and 5mCi of either ^{35}S, ^{32}P or ^{33}P can be used per experiment.

3. Areas designated for the use of radioisotopes include:

a. The two large laboratories at 597 Science Drive with four small work areas for specific functions: isotope lab, tissue culture room, microbiology lab and dark room.

b. The laboratory at 595 Science Drive with one small work area: microbiology room.

There will be no eating, no drinking, no storage of food or drink, and no applying cosmetics in these restricted areas.

b. *Apparel to wear, safety equipment to use:*

1. In addition to normal laboratory procedures, all personnel will wear lab coats and disposable gloves when working with any radioisotopes. The lab coats and gloves will be removed and left in a designated area when workers leave the restricted areas. Personal dosimeter badges will be worn by all isotope users (workers using ^{32}P see section "g" for further precautions).

2. Manipulation and storage of ^{32}P and ^{33}P radioisotopes will be performed behind 3/8 inch acrylic shields whenever possible.

3. All microcentrifugation of radiolabeled material will be performed in screw-top microcentrifuge tubes with rubber O-rings to minimize aerosol formation.

c. *Limitations and conditions on handling loose and liquid materials and equipment to use in working with them:*

1. All work with loose and liquid radioisotopes must be performed on surfaces protected by disposable absorbent spill paper or with a portable tray that can be washed with a decontaminating detergent and water after each use.

2. Loose and liquid radioisotopes are to be used only in designated areas by authorized users.

3. **All equipment and areas in which loose and liquid radioisotopes are used must be monitored after use and decontaminated if necessary.**

4. All pipetting of radioisotopes will be performed with remote pipetting devices.

5. All microcentrifugation of radiolabeled material will be performed in screw-top microcentrifuge tubes with rubber O-rings to minimize aerosol formation.

d. *Routine survey and monitoring procedures:*

1. Monthly wipe tests will be performed as specified under Novagen's license (item 10) by the Radiation Protection Officer or his designee.

2. In addition to monthly tests, each authorized user is responsible for monitoring and cleaning equipment and areas in which they work. ^{3}H and ^{14}C contamination will be monitored with wipe tests. An area 100 cm^2 should be wiped with a 3 cm x 3 cm square of tissue moistened with 70% ethanol. The tissue will be placed in a scintillation vial to which scintillation fluid will be added, followed by measurement of radioactivity in a scintillation counter. Any area showing more than 1000 cpm will be considered contaminated and will be cleaned with a commercially available decontaminating detergent until wipe tests show levels of 1000 cpm or less. ^{32}P, ^{33}P and ^{35}S contamination will be monitored with a Geiger Mueller survey meter (Model 900-E or Model GM2 from Research Products International Corp.).

e. *Movement of materials between rooms, corridors:*

1. All radioactive material will be transported in a box made of 3/8 inch acrylic plastic when it is necessary to transport radioactive materials between restricted areas.

f. *Requirements for storage and labeling of containers and identification of areas. Where and how contaminated articles and glassware are to be handled and stored:*

1. Equipment designated for use with radioisotopes will be marked with radioactive warning labels consisting of a yellow background with a purple or magenta three-bladed radiation symbol.

2. Areas of lab bench tops where radioisotopes are used will be labeled with radioactive warning labels. The bench tops will be covered with spill-proof absorbent paper or a portable tray that can be washed with decontaminating detergent and water.

3. Every effort will be made to use disposable plasticware and discard it with the radioactive waste. Contaminated glassware will be soaked in decontaminating detergent and rinsed well with running water until wipe tests or survey meter readings show levels of 1000 cpm or less.

g. *Personal monitoring devices to be used, where to obtain, recording exposure results or properly turning in devices for processing at appropriate intervals:*

1. Film badges will be obtained from Landauer (2 Science Rd., Glenwood, IL, 60429-1586) and returned to the company on a quarterly basis for analysis.

2. Film badges will be worn at waist or chest level by personnel working with ^{32}P, ^{33}P, or ^{35}S. Finger-type extremity monitors will be worn when using 1mCi or more of ^{32}P (see enclosed instructions for care and use of film badges).

h. *Waste disposal procedures, including limitations, and procedures for waste storage:*

1. ^{32}P solid waste will be stored in an appropriately labeled 3/8 inch acrylic container. As waste containers are filled, they will be sealed with tape and labeled with the date. After ten half-lives from the date the box is sealed (5 months for ^{32}P), the decayed ^{32}P solid waste will be discarded with other laboratory trash. See attached sheet.

2. ^{33}P solid waste will be stored in an appropriately labeled 3/8 inch acrylic container. As waste containers are filled, they will be sealed with tape and labeled with the date. After ten half-lives from the date the box is sealed (9 months for ^{33}P), the decayed ^{33}P solid waste will be discarded with other laboratory trash. See attached sheet.

3. The following procedures will be used for ^{35}S decay-in-storage waste:

a. Geiger-Mueller Monitors (Models 900-E and GM2, Research Products International, Mount Prospect, Illinois) will be used for surveying ^{35}S waste for decay-in-storage. The minimum detectable activity for the instruments is $2 \times 10^{-3}\mu Ci$ of ^{35}S. The final survey will be performed in a low radiation background area with all shielding removed.

b. The waste will be segregated to ensure that ^{35}S decay-in-storage is separately identified from interim storage wastes. The waste containers will be labeled as "^{35}S Decay-In-Storage" along with the yellow label which has a purple or magenta three bladed radiation symbol. The waste will be stored in a 3/8" thick acrylic container with a hinged lid. The lid will be locked at all times except to perform duties as listed below. Up to ten plastic bags of approximately 0.4 cubic feet each will be placed in the acrylic waste container. Each bag will be labeled with an identification number and the date placed in storage. Records for date placed in storage, date removed, and survey results will be kept for each plastic bag kept in the waste container. The ^{35}S waste will be inspected and surveyed monthly. Record sheets for ^{35}S decay-in-storage are attached. The maximum amount of ^{35}S for decay-in-storage will be 4 cubic feet and 20mCi.

c. The ^{35}S waste will be held for a minimum of 10 half-lives (30 months).

d. The decayed ^{35}S waste will not be disposed of as ordinary trash unless the final survey results are indistinguishable from background radiation, using the survey procedures discussed in Paragraph a, above, and radiation labels are obliterated or removed. Before disposal, the radioactive material in the container at the container surface will be monitored to determine that its radioactivity cannot be distinguished from background radiation levels. A record of each disposal of ^{35}S will be retained for 3 years. The record will include the date of the disposal, the date on which the ^{35}S was placed in storage, the radionuclide disposed of, the survey instrument used, the background dose rate, the dose rate measured at the surface of each waste container, and the name of the individual who performed the disposal.

4. ^{14}C and ^{3}H solid waste, such as pipet tips and microcentrifuge tubes, must be soaked in "Count-Off" or a comparable decontaminanting detergent and then rinsed with tap water.

5. Water-soluble liquid wastes will be diluted with water and released into the sanitary sewerage system if those amounts do not exceed the limits specified in 10 CFR Part 20, Subpart K-Waste Disposals, Sections 20.2001-20.2007.and table 3 of Appendix B to 10 CFR, Part 20, 20.1001-20.2402.

6. All ^{14}C, ^{3}H, and ^{35}S liquid waste must be miscible with water. No scientist at Novagen can generate ^{14}C, ^{3}H, or ^{35}S liquid waste that is not miscible with water. If you have to generate radioactive liquid waste that is not miscible with water, you must use a ^{32}P or ^{33}P radioisotope.

7. ^{32}P and ^{33}P liquid waste which is not miscible with water will be stored in appropriately marked carboys for 10 half-lives. The decayed waste will not be disposed of with other laboratory solvents unless the final survey results are indistinguishable from background radiation. Our hazardous waste disposal company is Waste Research and Reclamation Co., Inc. (5200 State Road 93, Eau Claire, WI 54701).

8. Remember that the total amount of isotope we may possess, as listed above, includes the amount in the waste containers.

i. *Records to be kept for use and disposal:*

1. Personnel monitoring records shall be preserved until the USNRC authorizes disposition.
2. Records of the results of surveys and monitoring shall be preserved for two years after the completion of the survey (see enclosed wipe test record and map of areas to be monitored).
3. Use and disposal records (see enclosed sample Radioactive Material Record) are to be maintained for at least two years after final use of the material.
4. Each authorized user will record the use and disposition of any isotopes used for each experiment on the appropriate Radioactive Material Record.

j. *Other instructions:*

1. *Specific areas for receiving, unpacking, and storage of isotopes:* Isotopes will be received at the front reception desk. Unpacking of isotopes will be done in the radioisotope room. Isotopes will be stored in the refrigerator/freezer in the radioisotope room or in the -70°C freezer or cold box in the production lab.
2. *Specific areas for storage of radioactive waste:* Radioactive waste will be stored in labeled containers (shielded containers for ^{32}P) in the radioisotope room.
3. *Specific areas designated for isotope use and restrictions associated with these areas (i.e. no eating, drinking, smoking, or applying cosmetics):* See above for description of restricted areas. There will be no eating, drinking, smoking, storage of food or beverages, or application of cosmetics in any of the laboratory areas.
4. *Specific procedures for containment and/or notification of appropriate personnel in the event of a spill:* In the event of a spill or leakage of radioactive material, the spread of the material will be limited by covering the spill with absorbent paper. Other people in the area of the spill will be notified of the problem. The personnel in the room will be monitored for contamination. The Radiation Protection Officer will be notified and he will oversee the cleanup and decontamination procedures. If immediate notification of the Radiation Protection Officer is not practical, refer to Chapter 6 of the Radiation Safety manual for appropriate cleanup procedures.

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^{35}S DECAY-IN-STORAGE RECORD
Individual Plastic Bag Record

Items 1-5 must be filled out when placing a plastic bag in the acrylic ^{35}S waste container for decay-in-storage.

1. Bag identification number:_____

2. Name of person who placed bag in storage:_____

3. Date placed in storage:_____

4. Quantity of ^{35}S (mCi) inside this bag on date placed in storage:_____mCi

5. Date of 10 half-lives for this bag(date placed in storage + 30 months):_____

Items 6-10 must be filled out when you dispose of the decayed material to ordinary trash. This date must be at the minimum of 10 half-lives, which is listed in number 5 above.

6. Date of disposal to ordinary trash:_____

7. Name of person performing final disposal:_____

8. Survey instrument used for final survey of bag:_____
Date instrument was last calibrated:_____

9. Background dose rate:_____dps

10. Dose rate measured at the surface of the plastic bag:_____dps

PLEASE NOTE THAT ^{35}S WASTE CANNOT BE DISPOSED OF AS ORDINARY TRASH UNLESS THE FINAL SURVEY RESULTS ARE INDISTINGUISHABLE FROM BACKGROUND RADIATION. RADIATION LABELS MUST BE OBLITERATED OR REMOVED BEFORE DISPOSAL!!!

^{32}P DECAY-IN-STORAGE RECORD
Individual Plastic Bag Record

Items 1-5 must be filled out when placing a plastic bag in the acrylic ^{32}P waste container for decay-in-storage.

1. Bag identification number:_____
2. Name of person who placed bag in storage:_____
3. Date placed in storage:_____
4. Quantity of ^{32}P (mCi) inside this bag on date placed in storage:_____mCi
5. Date of 10 half-lives for this bag(date placed in storage + 5 months):_____

Items 6-10 must be filled out when you dispose of the decayed material to ordinary trash. This date must be at the minimum of 10 half-lives, which is listed in number 5 above.

6. Date of disposal to ordinary trash:_____
7. Name of person performing final disposal:_____
8. Survey instrument used for final survey of bag:_____
   Date instrument was last calibrated:_____
9. Background dose rate:_____dps
10. Dose rate measured at the surface of the plastic bag:_____dps

PLEASE NOTE THAT ^{32}P WASTE CANNOT BE DISPOSED OF AS ORDINARY TRASH UNLESS THE FINAL SURVEY RESULTS ARE INDISTINGUISHABLE FROM BACKGROUND RADIATION. RADIATION LABELS MUST BE OBLITERATED OR REMOVED BEFORE DISPOSAL!!!

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^{33}P DECAY-IN-STORAGE RECORD
Individual Plastic Bag Record

Items 1-5 must be filled out when placing a plastic bag in the acrylic ^{33}P waste container for decay-in-storage.

1. Bag identification number:_____

2. Name of person who placed bag in storage:_____

3. Date placed in storage:_____

4. Quantity of ^{33}P (mCi) inside this bag on date placed in storage:_____mCi

5. Date of 10 half-lives for this bag(date placed in storage + 9 months):_____

Items 6-10 must be filled out when you dispose of the decayed material to ordinary trash. This date must be at the minimum of 10 half-lives, which is listed in number 5 above.

6. Date of disposal to ordinary trash:_____

7. Name of person performing final disposal:_____

8. Survey instrument used for final survey of bag:_____
Date instrument was last calibrated:_____

9. Background dose rate:_____dps

10. Dose rate measured at the surface of the plastic bag:_____dps

PLEASE NOTE THAT ^{33}P WASTE CANNOT BE DISPOSED OF AS ORDINARY TRASH UNLESS THE FINAL SURVEY RESULTS ARE INDISTINGUISHABLE FROM BACKGROUND RADIATION. RADIATION LABELS MUST BE OBLITERATED OR REMOVED BEFORE DISPOSAL!!!

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^{35}S DECAY-IN-STORAGE RECORD
Monthly Surveys and Inspections Record

Date	Person Doing Survey	Visual Inspection**	G-M Meter Used	Background Dose Rate	^{35}S Decay-In-Storage Dose Rate

**A visual inspection will include answering these questions:
1) Is the acrylic container for ^{35}S Decay-In-Storage locked?
2) Are the correct number of plastic bags present inside the container?
3) Are the identification numbers and dates correct on the plastic bags?
If the answer is yes to these three questions, then mark O.K. under the Visual Inspection column. If the answer is no to any of the questions, immediately notify the Radiation Protection Officer.

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^{35}S SOLID WASTE DISPOSAL
Record Sheet for Each Plastic Bag

NAME	DATE	NEN OR AMERSHAM LOT NUMBER	µCi DISPOSED

Identification Number:_____ Total for Decay-In-Storage: _____µCi

Name:_____ Date Placed in Decay-In-Storage: _____

NOVAGEN

RADIOACTIVE MATERIAL RECORD

Date Received: _____ RSR # _____

Supplier: _____ Catalog # _____ Lot # _____

Radionuclide: _____ Chemical: _____

Activity/unit: _____ ___ Ci X _____ (# units) = _____ Ci (Total Activity)

USE INFORMATION					DISPOSAL INFORMATION							
ACTIVITY (µCi or mCi)		VOLUME (µl or ml)		Date & Initials	WASTE TYPES AND ACTIVITY (mCi or µCi)						Waste Pickup #	Date & Initials
					Solid	Organic Liquid	Aqueous Liquid	Animal	Other	= TOTAL		
Removed	Remaining	Removed	Remaining									

Instructions:
1) Verify this order is correct and complete the information above; use this record sheet for this material only. You may add sheets to record use and disposal of any new samples, stocks, etc. labelled with this material.
2) Keep these records in a loose leaf binder or central area so they are available to all users.
3) Record use and disposal as they occur, and circle the appropriate volume and activity units. Use the "other" column to record any other disposal methods (e.g. decay, release to the sewer, etc.).
4) The total activity disposed should equal the total used, minus any activity remaining in samples, stocks, etc.
5) This record may be discarded two years after final use of the material

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5' End Labeling of Oligonucleotides with ^{32}P:

1. Assemble the following components in the order given in a screw-capped microcentrifuge tube:
   1.5 pmol dephosphorylated DNA 5' ends
   15µl [γ-^{32}P]dCTP (3,000Ci/mmole)
   10-20 units T4 polynucleotide kinase
   sterile H_2O to a final volume of 100µl

2. Incubate at 37° for 30 minutes.

3. Add 2 µl 0.5M EDT4. Add an equal volume of phenol/chloroform. Vortex briefly. Centrifuge at 12,000x g for 5 minutes.

5. Transfer the upper aqueous phase to a fresh tube, add 0.1 volume 2M NaCl and mix.

6. Add 2 volumes ethanol. Incubate at -20° for 30 minutes.

7. Centrifuge at 12,000x g for 10 minutes.

8. Discard the supernatant. Dry pellet.

9. Redissolve DNA pellet in 50µl TE.

Hybridizations of ^{32}P DNA and oligonucleotides:
(The labeled probes can be stored at -20°C for up to 2 weeks.)

1. Prewash an agarose gel blot in 3 XSSC, 0.1% SDS three times for 5 min each at room temp.

2. Incubate the blot in the same buffer at 60°C for 1.5 hr.

3. Prehybridization: remove the blot from the wash solution and let the excess solution drain; put into a heat-sealable pouch with hyb buffer (6 XSSC, 1% SDS, 0.05% NaPPi) and incubate at 37°C for 1hr.

4. Add the desired amount of probe (want about 5 x 10^6 incorporated cpm/ml) to 0.5ml hyb buffer and inject this solution into the hyb bag. Reseal and incubate at 37°C overnight.

5. Remove the blot from the hyb bag and wash three times for 5 min each in 6 XSSC, 0.05% NaPPi at room temp, then for 30 min at 37°C in the same solution. Let the excess liquid drain by placing on a piece of 3MM, cover with Saran, and expose to X-ray film.

6. Appropriate exposure times should be from 4-18 hr with screens.

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Synthesis of 32P Labeled RNA:

1. Assemble the following components in the order given in a screw-capped microcentrifuge tube:

4.0 µl 5X transcription buffer
2.0 µl 100nM DTT
20 units RNasin
4.0 µl solution of 2.5mM each ATP, GTP, and UTP
2.4 µl 100µM CTP
1.0 µl linear template DNA in H_2O or TE at a concentration of 0.2-1.0 mg/ml
5µl [α^{32}P]CTP (50µCi at 10mCi/ml)
1.0 µl SP6 RNA polymerase or T7 RNA polymerase (at 15-20 units/ml)
sterile H_2O to a final volume of 20µl

2. Incubate for 60 minutes at 37-40°.

DNA sequencing:

See attached literature.

^{35}S Translation:

See attached literature.

DNA sequencing

10. Centrifuge the supernatant again at 10,000 *g* for 5 min. Transfer the supernatant to another fresh tube.

11. Add RNase A to a final concentration of 20 μg/ml, and incubate at 37° for 20 min.

12. Add an equal volume of phenol–chloroform (1 : 1, saturated with 50 m*M* Tris–HCl at pH 8.0, 100 m*M* NaCl, 1 m*M* EDTA), mix by vortexing 30 sec, and centrifuge at 10,000 *g* for 30 sec. Transfer the aqueous phase to a fresh 1.5-ml tube.

13. Add 2.5 volumes of ethanol, mix, and incubate at −70° for 5 min.

14. Centrifuge at 10,000 *g* for 5 min. Remove the supernatant and rinse the pellet by adding 1 ml of prechilled 70% ethanol, mixing briefly, and centrifuging for 1 min. Carefully remove the supernatant and dry the pellet under vacuum.

15. Dissolve the pellet in 16 μl deionized water. Add 4 μl 4 *M* NaCl, mix, and then add 20 μl 13% polyethylene glycol (MW 8000). Mix well and incubate at 0° for 20 min.

16. Centrifuge at 10,000 *g* for 10 min. Remove supernatant and rinse and dry the pellet as in step 14.

17. Dissolve the DNA in 20 μl of deionized water. A yield of 1–3 μg should be expected.

Alkali Denaturation and Primer Annealing

1. Add 2 μl of 2 *N* NaOH, 2 m*M* EDTA to 20 μl of water containing 1–2 μg plasmid DNA in a siliconized, screw-capped microcentrifuge tube.

2. Incubate for 5 min at room temperature.

3. Neutralize by adding 3 μl sodium acetate (pH 5.2), and then add 7 μl deionized water. Mix, add 75 μl ethanol, mix again, and chill for 5 min at −70°.

4. Centrifuge at 10,000 *g* for 5 min, remove the supernatant, and rinse the pellet with 200 μl prechilled 70% ethanol. Dry the final pellet under vacuum.

5. To the dry pellet, add 6 μl deionized water, 1 μl of 10× buffer (100 m*M* Tris–HCl at pH 7.5, 500 m*M* NaCl for Klenow DNA polymerase; 340 m*M* Tris–HCl at pH 8.3, 500 m*M* NaCl, 60 m*M* $MgCl_2$, 50 m*M* dithiothreitol for reverse transcriptase), and 3 μl of the appropriate promoter primer (10 ng/μl). Anneal at 37° for 15–20 min.

Sequencing Reactions

1. Prepare four 1.5-ml screw-capped microcentrifuge tubes labeled C, A, T, and G for each set of sequencing reactions. Add 3 μl of the appropriate nucleotide mix to each tube.

Klenow DNA polymerase mixes
ddCTP mix: 66 μM ddCTP, 1.66 μM dCTP, 33 μM dTTP, 33 μM dGTP
ddATP mix: 300 μM ddATP, 33 μM dCTP, 33 μM dTTP, 33 μM dGTP
ddTTP mix: 117 μM ddTTP, 33 μM dCTP, 1.66 μM dTTP, 33 μM dGTP
ddGTP mix: 66 μM ddGTP, 33 μM dCTP, 33 μM dTTP, 1.66 μM dGTP
(all mixes contain 10 mM Tris–HCl at pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol)

Reverse transcriptase mixes
ddCTP mix: 100 μM ddCTP, 250 μM dCTP, 250 μM dTTP, 250 μM dGTP
ddATP mix: 3.6 μM ddATP, 250 μM dCTP, 250 μM dTTP, 250 μM dGTP
ddTTP mix: 200 μM ddTTP, 250 μM dCTP, 250 μM dTTP, 250 μM dGTP
ddGTP mix: 50 μM ddGTP, 250 μM dCTP, 250 μM dTTP, 250 μM dGTP
(all mixes contain 34 mM Tris–HCl at pH 8.3, 50 mM NaCl, 6 mM $MgCl_2$, 5 mM dithiothreitol)

2. Add 5 units of Klenow DNA polymerase or 5 units of reverse transcriptase to the annealing mixture (step 5, previous section).

3. Add 4 μl [α-^{32}P]dATP (10 mCi/ml; >400 Ci/mmol) or 5 μl [α-^{35}S]dATP (10 mCi/ml; >500 Ci/mmol) to the annealing mixture. Pipet up and down a few times to mix.

4. Add 3 μl of the primer–template–enzyme–label mixture to each of the nucleotide mixes and incubate at 37° for 15 min (20 min if using [α-^{35}S]dATP). Incubate at 42° for reverse transcriptase.

5. Add 1 μl of chase solution to each tube and incubate at 37° for another 15 min (42° for reverse transcriptase). (Chase solution: 2 mM each of dCTP, dATP, dTTP, and dGTP in the same buffer as the nucleotide mixes.)

6. Stop the reactions by adding 5 μl of 90% formamide containing 20 mM EDTA, 0.3% bromphenol blue, and 0.3% xylene cyanole. Mix well.

7. Heat the reactions at 70° for 3 min before loading the sequencing gel. Load 2.5 μl of each reaction. If multiple loading is desired to increase the amount of sequence read, the samples should be heated at 70° for 3 min immediately before each load to prevent possible renaturation.

The nucleotide mixes in step 1 have been optimized for using labeled dATP of a specific activity of 400–500 Ci/mmol, which results in a final dATP concentration of about 2 μM. If higher specific activities are used, the mixes should be supplemented with unlabeled dATP to achieve proper nucleotide ratios. Using a standard 6 or 8% sequencing gel with two loads, it is normal to read 200–250 bases for ^{32}P and 300–350 bases for ^{35}S from each end of the insert with this method. When the size of the insert is too large (>500 bp) to allow the complete sequence to be determined from both ends using both promoter primers, a series of progressive unidirectional deletion subclones can usually be constructed (depending on available restriction sites) using exonuclease III by the method described by Henikoff[16] or as described in this volume [57].

In summary, sequencing denatured plasmid DNA with either Klenow DNA polymerase or reverse transcriptase yields results comparable to those obtained from single-stranded M13 DNA with the corresponding enzymes. The method allows sequencing of both strands of a single plasmid isolate when a variety of vectors are used for recombinant constructions. The use of both sequencing enzymes provides an additional (or alternative) way to confirm, clarify, or extend a given sequence without additional template preparation.

[*Editors' Note*. Stephen A. Saxe (Laboratory of Cellular and Developmental Biology, National Institutes of Health) has modified the protocol for dideoxy sequencing of DNA templates to optimize the reading of G stretches. Others have previously suggested that reactions with Klenow fragment may be incubated at 50° (rather than 37°) but ambiguities are often still observed; the use of reverse transcriptase at 42° instead of Klenow fragment is not optimal for reading long AT stretches. Saxe suggests using 5 units of Klenow enzyme (rather than 1 unit) for *each* reaction with incubations at 37°. Excess Klenow fragment clearly promotes the reading of both GC and AT stretches.]

[16] S. Henikoff, *Gene* **28**, 351 (1984).

Rabbit Reticulocyte Lysate, Nuclease Treated

IV. Rabbit Reticulocyte Lysate, Nuclease Treated

A. Description

The rabbit reticulocyte translation system plays an important role in the identification of mRNA species, the characterization of their products, the investigation of transcriptional and translational control, and the cotranslational processing of secreted proteins by the addition of microsomal membranes to the translation reaction. Reticulocyte lysate is prepared from New Zealand White rabbits injected with phenylhydrazine using a standard protocol (9) which ensures reliable and consistent reticulocyte production in each lot. The reticulocytes are purified to remove contaminating cells which could otherwise alter the translational properties of the final extract. After the reticulocytes are lysed, the extract is treated with micrococcal nuclease to destroy endogenous mRNA and thus reduce background translation to a minimum. The lysate contains the cellular components necessary for protein synthesis: tRNA; rRNA; amino acids; and initiation, elongation, and termination factors. Reticulocyte lysate is further optimized for mRNA translation by adding:

- An energy generating system consisting of pretested phosphocreatine kinase and phosphocreatine.
- A mixture of tRNAs to expand the range of mRNAs which can be translated.
- Hemin to prevent inhibition of initiation.
- Potassium acetate and magnesium acetate at a level recommended for the translation of most mRNA species.

Processing events such as signal peptide cleavage and core glycosylation can be examined by adding canine microsomal membranes to a standard translation reaction (10). See pgs. 127-128. (Canine Pancreatic Microsomal Membranes) for details.

B. Translation Procedure

Materials Required

The following reagents are not included with the rabbit reticulocyte and wheat germ extract translation systems and must be supplied by the researcher:

Double-distilled, RNase-free water

RNasin® ribonuclease inhibitor

Isotopically labeled amino acids, typically ^{35}S-methionine, ^{35}S-cysteine, ^{3}H-leucine, ~~or ^{14}C-leucine~~.

1N NaOH/2% H_2O_2 for hydrolysis of aminoacyl tRNAs. Make up 100ml and store at room temperature.

25% (w/v) Trichloroacetic acid (TCA)/2% casamino acids (or 2% bovine serum albumin) for precipitation of translation products. Make up 500ml and store at 0°C.

5% (w/v) TCA for washing of precipitated polypeptides. Make up 500ml and store at 0°C.

Acetone

Reagents for scintillation counting and fluorography

Reagents for performing SDS gel electrophoresis (see pg. 131).

The following equipment will be needed to perform and analyze translation reactions *in vitro*:

Polypropylene microcentrifuge tubes, 0.5ml and 1.5ml capacity

Adjustable pipettors and disposable, RNase-free plastic tips suitable for pipetting 1 to 200µl

Plastic gloves

Constant temperature bath (for 25°C, 37°C, and 65°C)

Vortex mixer

Microcentrifuge

GF/A glass fiber filters and a vacuum filtration apparatus

Scintillation counter and vials

Slab gel electrophoresis and gel drying apparatus

35 S Translation

Promega

Rabbit Reticulocyte Lysate, Nuclease Treated

(continued)

About 2 hours should be allowed to perform the translation reaction. The SDS gel can conveniently be set up and cast (1 hour) during this time. Allow 2 hours to perform TCA precipitation and preparation of filters for scintillation counting. Two hours should also be allowed to load and run the SDS gel, with an additional 2 hours for the optional staining and destaining procedures.

1. Remove reagents from storage and allow to thaw slowly on ice.
2. Heat the template mRNA at 67°C for 10 minutes and immediately cool on ice. This increases the efficiency of translation, especially of GC-rich mRNA, by destroying local regions of secondary structure.

^{35}S-Methionine

35µl nuclease treated lysate
7µl H_2O
1µl RNasin ribonuclease inhibitor (at 40u/µl)
1µl 1mM amino acid mixture (minus methionine)
2µl RNA substrate in H_2O (see Note 2, pg. 122)
4µl ^{35}S-methionine (1,200Ci/mmole) at 10mCi/ml
50µl [Final ^{35}S-methionine concentration = 0.8mCi/ml]

^{3}H-Leucine

35µl nuclease treated lysate
6µl H_2O
1µl RNasin ribonuclease inhibitor (at 40u/µl)
1µl 1mM amino acid mixture (minus leucine)
2µl RNA substrate in H_2O (see Note 2, pg. 122)
5µl ^{3}H-leucine (100-200Ci/mmole) at 5mCi/ml
50µl [Final ^{3}H-leucine concentration= 0.5mCi/ml]

^{14}C-Leucine

35µl nuclease treated lysate
6µl H_2O
1µl RNasin ribonuclease inhibitor (at 40u/µl)
1µl 1mM amino acid mixture (minus leucine)
2µl RNA substrate in H_2O (see Note 2, pg. 122)
5µl ^{14}C-leucine (300mCi/mmole) at 50µCi/ml
50µl [Final ^{14}C-leucine concentration = 5µCi/ml]

to the label being used (see Standard Reactions, below) in a 0.5ml polypropylene microcentrifuge tube. Gently mix the lysate upon addition of each component. If necessary, spin briefly in a microcentrifuge to return the sample to the bottom of the tube. (**Note:** We recommend including a control reaction containing no added mRNA. This allows measurement of any background incorporation of labeled amino acids.)

4. Incubate the reticulocyte translation reactions at 30°C for 60 minutes.
5. Analyze the results of translation. Procedures are provided for incorporation assays (pg. 129) and gel analysis of translation products (pg. 130).

^{35}S-Cysteine

35µl nuclease treated lysate
6µl H_2O
1µl RNasin ribonuclease inhibitor (at 40u/µl)
1µl 1mM amino acid mixture (minus cysteine)
2µl RNA substrate in H_2O (see Note 2, pg. 122)
5µl ^{35}S-cysteine (1,200Ci/mmole) at 10mCi/ml
50µl [Final ^{35}S-cysteine concentration = 1mCi/ml]

^{35}S-Methionine and ^{3}H-Leucine

34µl nuclease treated lysate
6µl H_2O
1µl RNasin ribonuclease inhibitor (at 40u/µl)
1µl 1mM amino acid mixture (minus methionine and leucine)
2µl RNA substrate in H_2O (see Note 2, pg. 122)
3µl ^{35}S-methionine (1,200Ci/mmole) at 10mCi/ml
3µl ^{3}H-leucine (100-200Ci/mmole) at 5mCi/ml
50µl [Final ^{35}S-methionine concentration = 0.6mCi/ml, ^{3}H- leucine concentration = 0.3mCi/ml]

TCA Protein Precipitation Assay for Amino Acid Incorporation

VIII. TCA Protein Precipitation Assay for Amino Acid Incorporation

A. TCA Precipitation Procedure

The following protocol is used for the determination of labeled amino acid incorporation into protein during a typical translation reaction.

1. Vortex the translation reaction gently prior to removing each 2µl aliquot to be analyzed for incorporation.
2. Add the 2µl aliquot to 248.0µl 1N NaOH/2% H_2O_2 solution in a 1.5ml microcentrifuge tube and mix. The NaOH hydrolyzes aminoacyl tRNAs and thus prevents labeled tRNA from being included in the incorporation calculation. Hydrogen peroxide removes the red color from the reaction, thus preventing any quenching during scintillation counting. The peroxide may be left out when analyzing wheat germ translation products.
3. After all aliquots have been taken from the translation reaction, incubate them at 37°C for 10 minutes.
4. At the end of the 10 minute incubation, add 1.0ml of ice-cold 25% TCA/2% casamino acids to precipitate the translation products. (The casamino acids act as a carrier.) Incubate on ice for 30 minutes.
5. Collect the precipitate by filtering under vacuum on Whatman GF/A glass fiber filters. Wet the filter paper with a small amount of ice-cold 5% TCA. Filter the reaction and rinse the filter 3 times with 3ml of ice-cold 5% TCA. Rinse once with 1-3ml acetone. Allow the filter to dry at room temperature.
6. For determination of ^{35}S or ^{14}C incorporation, put the filter in 1-3ml of an appropriate scintillation mixture, invert to mix, and count. For ^{3}H add the same amount of scintillation mixture, invert, but leave the scintillation vials in the dark for 30 minutes at room temperature prior to counting.
7. To determine total counts present in the translation reaction, spot a 2-5µl aliquot of the reaction mix directly onto the filter paper. Count in a liquid scintillation counter as in step 6.
8. To determine background counts, remove 2-5µl from a reaction containing no mRNA and proceed as described in steps 2-6.

B. Sample Calculation to Determine the Efficiency of Translation

1. Total radioactivity/µl is first determined (see step A.7).

$$\frac{\text{amt. of radioactivity determined}}{\mu\text{l of reaction filtered}}$$

Example: $\frac{4 \times 10^6 \text{ cpm}}{2\mu\text{l}} = 2 \times 10^6 \text{ cpm}/\mu\text{l}$

2. Total radioactivity in the translation reaction (typical volume is 50µl)

$$\frac{\text{amt. of radioactivity determined}}{\mu\text{l of reaction filtered}} \times \text{total reaction vol.}$$

Example: $2 \times 10^6 \text{ cpm}/\mu\text{l} \times 50\mu\text{l} = 1 \times 10^8 \text{ cpm}$.

3. Number of TCA precipitable counts (cpm incorporated into protein) of an aliquot of the translation reaction (see step A.5).

$$\frac{\text{number of counts incorporated into protein}}{\mu\text{l of reaction TCA precipitated}}$$

Example: $\frac{1 \times 10^6}{2\mu\text{l}} = 5 \times 10^5 \text{ cpm}/\mu\text{l}$

4. Total number of counts incorporated into protein in a standard reaction (volume = 50µl).

$$\frac{\text{number of counts incorporated into protein}}{\mu\text{l of reaction TCA precipitated}} \times \text{total vol.}$$

Example: $5 \times 10^5 \text{cpm}/\mu\text{l} \times 50\mu\text{l} = 2.5 \times 10^7 \text{ cpm}$

5. Percent incorporation

$$\frac{\text{total radioactivity incorporated into protein} \times 100}{\text{total radioactivity in the reaction}}$$

Example: $\frac{2.5 \times 10^7 \text{cpm} \times 100}{1 \times 10^8 \text{cpm}} = 25\% \text{ incorp.}$

SDS Gel Analysis of Translation Products

IX. SDS Gel Analysis of Translation Products

The most widely applicable and versatile method for analysis of cell-free translation products synthesized from mixtures of RNAs is polyacrylamide slab gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (SDS) and a discontinuous buffer system. The separating gel at a concentration of 15% acrylamide gives good separation of peptide mixtures between 20,000 and 100,000 molecular weight (MW), with peptides between 55,000 and 60,000 MW migrating halfway down the length of the gel.

A. Sample Preparation

1. Once the translation reaction is complete (or at any desired time point), remove a 5µl aliquot and add it to 20µl SDS sample buffer (the rest of the reaction may be stored at –20°C). Add β-mercaptoethanol to the SDS sample buffer just before use.
2. Cap the tube and heat for 2 minutes at 100°C.
3. A small aliquot of the sample (5µl) can then be loaded on an SDS gel or stored at –20°C. Because a large fraction of the label is incorporated into protein, it is not necessary to separate labeled polypeptides from free amino acids by acetone precipitation.
4. Typically, electrophoresis is carried out at a constant current of 15mA in the stacking gel and 30mA in the separating gel. Electrophoresis is usually performed until the bromphenol blue dye front has run off the bottom of the gel. Since this dye front also contains the free labeled amino acids, disposal of unincorporated label may be easier if the gel is stopped while the dye front remains in the gel.

Note: Gel banding patterns may be improved by loading unlabeled reticulocyte lysate or wheat germ extract in the lanes adjacent to radioactive sample lanes.

B. Preparation of SDS-Polyacrylamide Gels

Formulations for preparing separating and stacking minigels are provided below. Pour the separating gel mix into the assembled gel plates, leaving sufficient space at the top for the stacking gel to be added later. Gently overlay the gel mix with 0.1% SDS. After polymerization (10-15 minutes), remove the overlay and rinse the surface of the separating gel first with water to remove any unpolymerized acrylamide and then with a small volume of stacking gel mix. Fill the remaining space with stacking gel mix and insert the comb immediately. After the stacking gel has polymerized, (30-45 minutes), remove the comb and rinse the wells to remove unpolymerized acrylamide.

	Volume for different % acrylamide				
Separating Gel Component	**8.5%**	**10%**	**12.5%**	**15%**	**17.5%**
H_2O	3.50ml	3.10ml	2.50ml	1.80ml	1.20ml
Acrylamide solution	2.00ml	2.40ml	3.00ml	3.70ml	4.30ml
Separating gel buffer	1.9ml	1.9ml	1.9ml	1.9ml	1.9ml
10% Ammonium persulfate	112µl	112µl	112µl	112µl	112µl
TEMED	5µl	5µl	5µl	5µl	5µl

Technical Services:
800-356-9526

SDS Gel Analysis of Translation Products

(continued)

C. SDS-Polyacrylamide Gel Solutions

(Recipes are sufficient for the preparation of a slab minigel 0.75mm thick, 100mm x 70mm.)

Separating gel buffer (per 100ml)
store at room temperature:
18.17g Tris base
4ml 10% SDS
pH to 8.8 with 12N HCl
to a final volume of 100ml with H_2O

Acrylamide (30%) (per 100ml)
store at 4°C:
30g acrylamide
0.8g bisacrylamide
to a final volume of 100ml with H_2O

Stacking gel buffer (per 100ml)
store at room temperature:
6.06g Tris-base
4ml 10% SDS
pH to 6.8 with 12N HCl
to a final volume of 100ml with H_2O

SDS sample buffer (per 10ml)
store at room temperature:
1.0ml glycerol
2.0ml 10% SDS
2.0mg bromophenol blue
1.25ml stacking gel buffer
to a final volume of 10ml with H_2O.
Just before use, add 1µl β-mercaptoethanol per 20µl of sample buffer.

Stacking Gel Component	Volume
H_2O	1.0ml
Stacking gel buffer	444µl
Acrylamide (30%)	300µl
Ammonium persulfate (10%)	28µl
TEMED	5µl

D. Staining of SDS Gels

After electrophoresis, protein bands in the gel may be visualized by staining with Coomassie blue dye. Coomassie staining, however, is usually not sensitive enough to detect translation products, and thus need not be performed before analyzing gel results by fluorography.

1. Stain with gentle agitation until the dye has penetrated the gel (approximately 1 hour).
2. Dye that is not bound to protein is removed by transferring the gel to destaining solution. Add a wad of Kim-wipes or similar tissues to absorb excess stain and gently agitate in this solution until bands are clearly visible (changing the solution may be required). Note that in this solution the gel will shrink somewhat.
3. Place the gel in swelling buffer for 2-6 hours. This allows the gel to return to its original size.
4. Cut a sheet of Whatman 3MM paper a little larger than the gel itself. Place this under the gel once swelling has been completed. Transfer the gel to a vacuum gel drier. Place plastic wrap over the gel and dry for 1 hour at 60°C followed by 1 hour at 80°C.

Staining and Destaining Solutions

Protein stain (per liter)
store at room temperature:
500ml 95% EtOH
100ml glacial acetic acid
400ml H_2O
2.0g Coomassie brilliant blue R250

Destaining solution (per liter)
store at room temperature:
500ml methanol
75ml glacial acetic acid
425ml H_2O

Swelling solution (per liter)
store at room temperature:
70ml glacial acetic acid
20ml glycerol
910ml H_2O

Translation *in vitro*

SDS Gel Analysis of Translation Products

(continued)

E. Fluorography

Following electrophoresis, labeled protein bands in gels may be visualized by autoradiography or fluorography. Fluorography dramatically increases the sensitivity of detection of ^{35}S, ^{14}C and ^{3}H labeled proteins, and is recommended for the analysis of translation *in vitro* products. Autoradiography is sufficiently sensitive to detect ^{35}S-labeled BMV RNA translation products using an 8-12 hour exposure to film (Kodak X-OMAT AR).

The increased detection sensitivity of fluorography is obtained by infusing an organic scintillant into the gel. The scintillant converts the emitted energy of the isotope to visible light and so increases the proportion of energy which may be detected by X-ray film. Commercial reagents are available which can conveniently be used for fluorographic enhancement of signal.

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X. References:

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Technical Literature

XI. Additional Translation *in vitro* Literature Available from Promega

Manual

Translation *in vitro* Technical Manual

Technical Bulletins

003 Nuclease Treated Lysate, Rabbit Reticulocyte; User Information

004 Untreated Lysate, Rabbit Reticulocyte; User Information

035 Wheat Germ Extract; User Information

042 Canine Pancreatic Microsomal Membranes

***Promega Notes* Articles**

Issue	Title of Article
9	Wheat germ extract
9	Translation in wheat germ extract of *in vitro* synthesized mRNA
11	Assaying protein translocation across the endoplasmic reticulum membrane (microsomes)

Promega

CARE AND USE OF YOUR RADIATION DOSIMETER

The radiation dosimeter, or badge, is a useful tool in radiation safety if you use it properly. The instructions below will help you get the most useful information from your badge.

Your dosimeter is not a "film badge". Instead of film, it contains radiation-sensitive crystals called thermoluminescent dosimeters (TLD's). It will measure your radiation exposure from sources located outside your body.

The badge doesn't protect you from radiation. But it can tell you how well you're protecting yourself from radiation. If your badge readings are higher than those people doing work similar to yours, or if your exposure increases when you change procedures, you'll know you should review your radiation protection procedures.

Anytime you have questions about your badge, ask Novagen's Radiation Safety Officer.

WEARING YOUR BADGE

1. Never wear anyone else's badge. Never let anyone else wear your badge. If there is a high reading, you won't know who was exposed.

2. Wear your badge between your collar and waist in the place where the exposure will be highest.

3. If you are using a ring badge (you must wear one when using 1mCi or more of ^{32}P!), wear your ring badge on the hand that would receive the most exposure. Wear it with the label toward the radiation source so you'll know what your maximum exposure is. Also, wear the ring badge inside your glove. But be careful - the ring can sometimes be pulled off your finger as you remove the glove.

IN CASE OF EMERGENCY

4. If you believe you or your badge may have been overexposed, tell Novagen's Radiation Safety Officer IMMEDIATELY. The Radiation Safety Officer will tell you what to do.

GENERAL HANDLING PROCEDURES

5. Never intentionally expose your badge to a radiation source.

6. Exposure to high temperatures can result in reduced exposure readings. Don't store your dosimeter near a source of intense heat or run it through a clothes dryer.

ADMINISTRATIVE PROCEDURES

7. If you lose your badge tell the badge leader.

8. If there are mistakes in the information printed on the dosimeter label or exposure reports, tell the badge leader immediately.

9. The date on your badge is the beginning of the badging period. At the end of the period, the badge group leader will take your old dosimeter and give you a new one.

10. The most current exposure report is posted in the office area. Other exposure reports are stored in the Radiation Safety File Cabinet and are available from the Radiation Safety Officer.

11. For questions about or changes in your dosimeter, ask the badge leader or the Radiation Safety Officer.

Radiation Safety Exam

Name: Date:

Social Security Number:

Birth Date:

List all radionuclides you will be using:

1. Who is Novagen's Radiation Protection Officer?

2. _____rays and_____rays are high energy electromagnetic waves similar to light.

3. After two half lives of any given radionuclide, what fraction of the original activity will be remaining?

4. The unit of absorbed dose (energy deposited per gram of tissue) is the _____.

5. The basic unit used to measure activity is the _____ which is equal to 3.7 x 10^{10} disintegrations per second.

6. The basic unit of measurement of exposure is _____., the quantity that expresses the ionization produced by gamma or X-rays in air

7. List the three products of radioactive decay in the order of most penetrating to least penetrating.

8. Biological damage due to radioactive materials is caused by ____ _____, which is the separation of electrons from atoms.

9. List four low energy beta emitters which are not an external radiation hazard.

10. Do people working with the above low energy beta emitters need to wear a film badge?

11. List two possible natural causes responsible for the continual occurrence of chromosome damage.

12. List three radionuclides which are external hazards if improperly shielded.

13. During what part of a pregnancy is the fetus most radiosensitive?

14. What is the NCRP's maximum dose recommendation for an unborn child?

15. What is the average accumulated dose due to natural background (excluding medical exposures) for an individual living in the United States for twelve years?

16. Radiation exposure can be reduced by increasing or decreasing what three factors? (specify increase or decrease with each factor)

17.Moving twice as far away from a radioactive source reduces the dose by a factor of ___.

18. Four methods of radioactive waste disposal are:

19. In general, will objects placed near a sealed source of radioactivity become radioactive?

20.Whom would you notify in the event of a radiation emergency?

21. In what part of a radionuclide laboratory may food and beverages be stored, prepared or consumed?

22.What is the procedure for ordering isotopes at Novagen?

23. How often must equipment and areas be monitored?

24. What level of radioactivity requires decontamination?

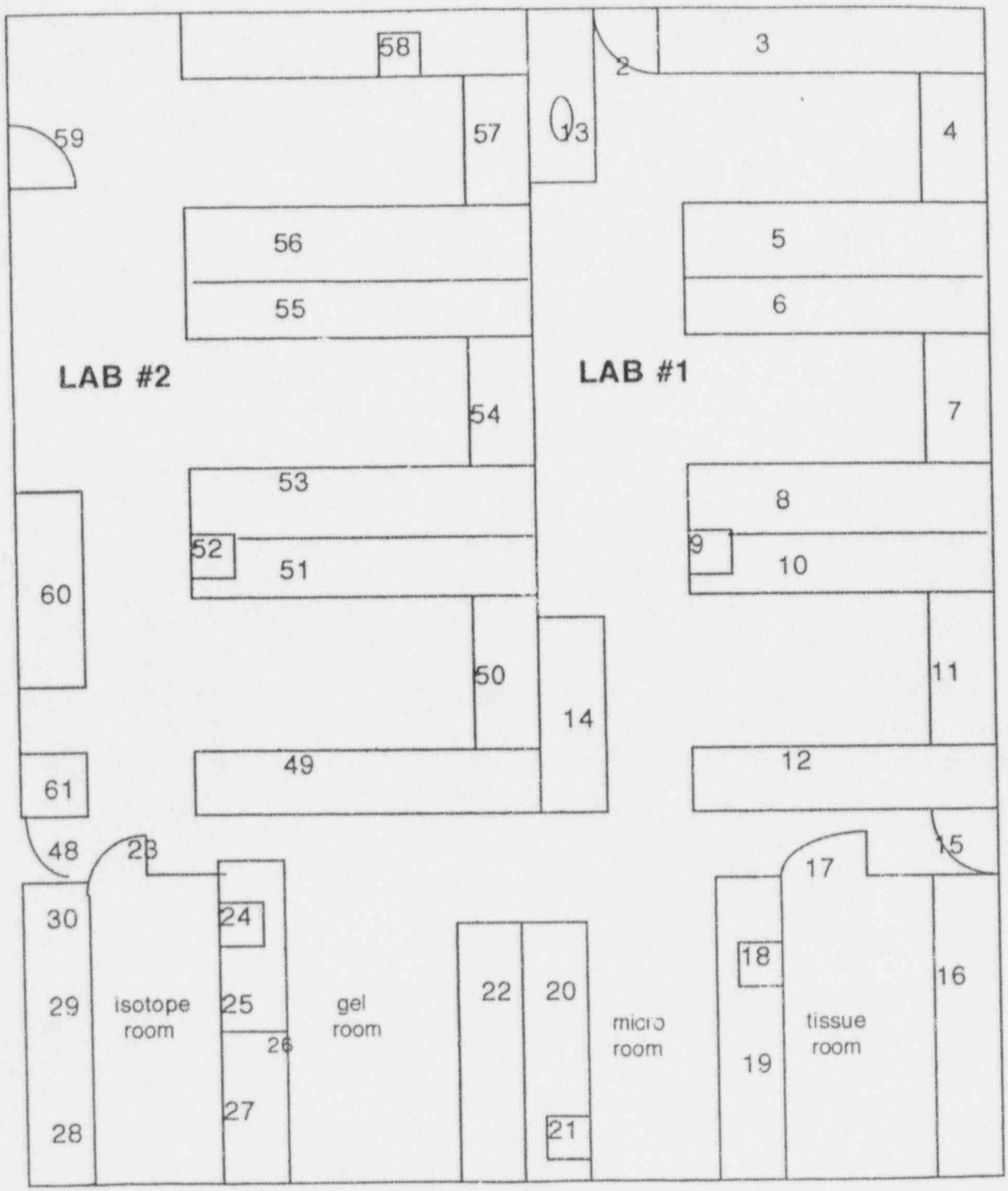
I have read and understood the handbook "Rdiation Safety for Laboratory Technicians" and completed this examination.

Signature: _____ .

Date: _____ .

Wipe test map for 597 Science Drive Laboratory

Surveyor's Name:
Date:



Please attach cpm data.
Any area showing more than 1000 cpm, must be cleaned until wipe tests show levels of 1000 cpm or less.

Wipe test for 597 Science Drive Laboratory

area or equipment wiped (refer to map):

1. background

LAB 1
2. door to front office
3. counter top
4. counter top
5. counter top
6. counter top
7. counter top
8. counter top
9. sink
10. counter top
11. counter top
12. counter top
13. microcentrifuge in cold box
14. -70°C freezer door handle

TISSUE ROOM
15. door
16. counter top
17. door to tissue room
18. sink
19. counter top

MICRO ROOM
20. counter top
21. microwave door handle

GEL ROOM
22. counter top

ISOTOPE ROOM
23. door to isotope room
24. sink
25. counter top
26. sink under the hood
27. counter top under the hood
28. counter top
29. counter top
30. counter top
31. ^{3}H solid waste container
32. ^{3}H liquid waste container
33. ^{32}P solid waste container
34. ^{32}P solid waste container
35. ^{35}S solid waste container
36. ^{35}S solid waste container
37. p-20 pipetman *
38. p-200 pipetman *
39. p-1000 pipetman *
40. microcentrifuge - interior
41. microcentrifuge - exterior
42. Geiger counter *
43. 37°C heat block *
44. 70°C heat block *
45. vortexer *
46. PCR machine
47. Lauda water bath

LAB 2
48. door to corridor
49. counter top
50. counter top
51. counter top
52. sink
53. counter top
54. counter top
55. counter top
56. counter top
57. counter top
58. sink
59. door to corridor
60. cold box door handle
61. -20°C freezer door handle

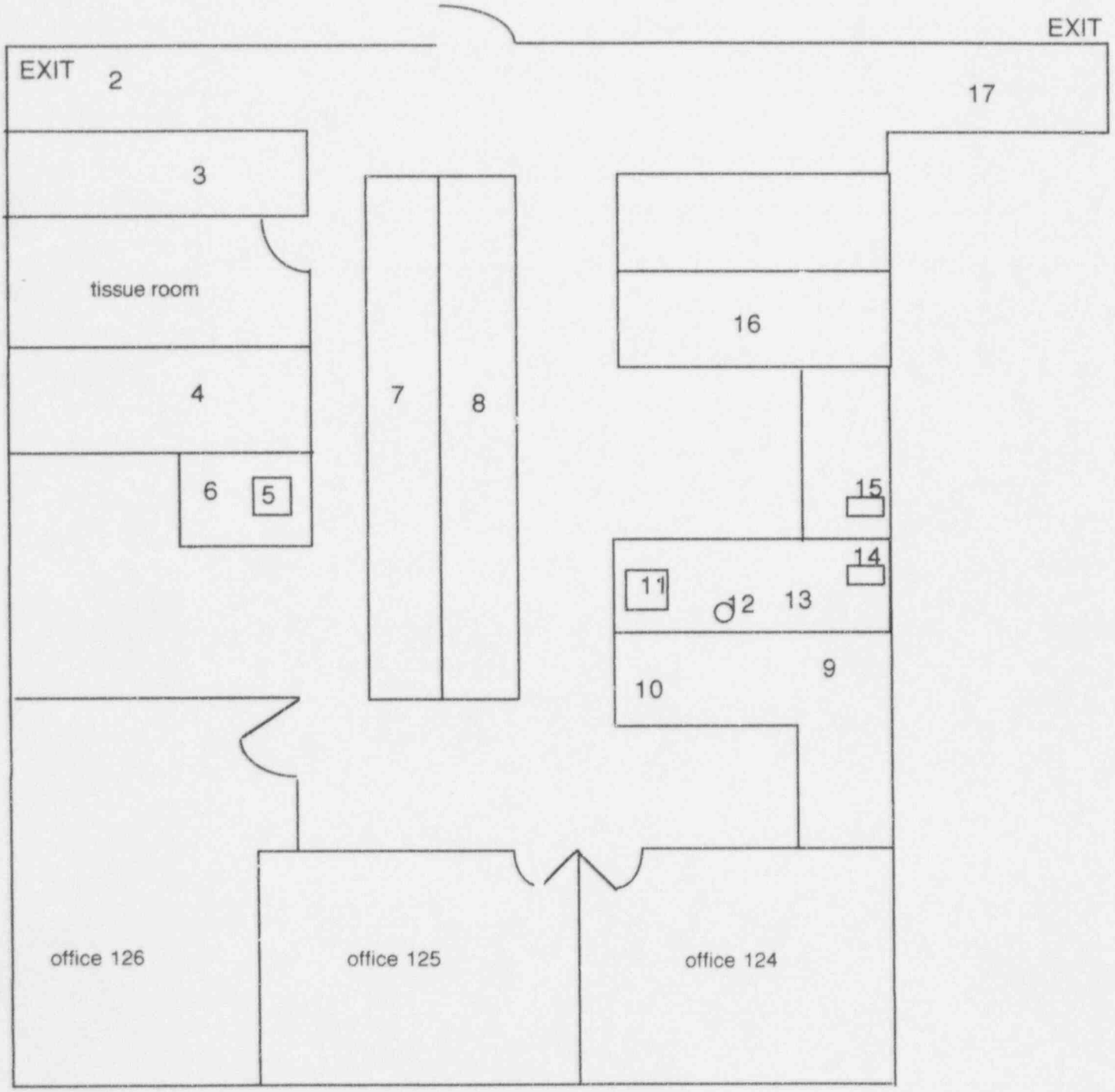
* = equipment which may move around to another location

Novagen

Radiation Safety

Wipe test map for 595 Science Drive Laboratory

Surveyor's Name:
Date:



Please attach cpm data.
Any area showing more than 1000 cpm, must be cleaned until wipe tests show levels of 1000 cpm or less.

Wipe test for 595 Science Drive Laboratory

area or equipment wiped (refer to map):

1. background
2. floor by exit to parking lot
3. bench on left in tissue culture room
4. bench on right in tissue culture room
5. sink by centrifuge
6. countertop by centrifuge
7. right side of island bench
8. left side of island bench
9. bench near corner office
10. bench near sink
11. sink
12. vortexer *
13. bench
14. 70°C heat block *
15. 80°C heat block *
16. bench
17. floor by exit

* = equipment which may move around to another location

DEC 0 9 1996

Robert C. Mierendorf, Ph.D
Vice President and General Manager
Novagen, Inc.
601 Science Drive
Madison, WI 53711

Dear Dr. Mierendorf:

Enclosed is Amendment No. 04 renewing your NRC Material License No. 48-26067-01 in accordance with your request.

Please review the enclosed document carefully and be sure that you understand all conditions. If there are any errors or questions, please notify the U.S. Nuclear Regulatory Commission, Region III office at (630) 829-9887 so that we can provide appropriate corrections and answers.

Please note that we included the facilities at both your 597 and 601 Science Drive addresses in Condition 10 of the enclosed document. Please note that we cannot authorize you to release the facilities located at 597 Science Drive for unrestricted use until we have received and reviewed a close-out survey for that location. Please refer to the enclosed document entitled "Required Information for the Decommissioning and Termination of Licensed Facilities" for the information that must be included in your close-out survey for the facilities located at 597 Science Drive.

In addition, your application referenced an erroneous license number for the University of Wisconsin-Madison (UWM), whom you indicated would be providing your survey instrument calibrations. Note Condition 13. of your license authorizes UWM (under the appropriate license number) or any other licensed organization to provide calibration services on an annual basis.

Please be advised that your license expires at the end of the day, in the month, and year stated in the license. Unless your license has been terminated, you must conduct your program involving byproduct materials in accordance with the conditions of your NRC license, representations made in your license application, and NRC regulations. In particular, note that you must:

1. Operate in accordance with NRC regulations 10 CFR Part 19, "Notices, Instructions and Reports to Workers; Inspections," 10 CFR Part 20, "Standards for Protection Against Radiation," and other applicable regulations.

2. Notify NRC, in writing, within 30 days:

   a. When the Radiation Safety Officer permanently discontinues performance of duties under the license or has a name change; or

   b. When the licensee's mailing address changes (no fee is required if the location of byproduct material remains the same).

3. In accordance with 10 CFR 30.36(b) and/or license condition, notify NRC, promptly, in writing, and request termination of the license when you decide to terminate all activities involving materials authorized under the license.

4. Request and obtain a license amendment before you:

   a. Change Radiation Safety Officers;

   b. Order byproduct material in excess of the amount, or radionuclide, or form different than authorized on the license;

   c. Add or change the areas of use or address or addresses of use identified in the license application or on the license; or

   d. Change ownership of your organization.

5. Submit a complete renewal application with proper fee or termination request at least 30 days before the expiration date of your license. You will receive a reminder notice approximately 90 days before the expiration date. Possession of byproduct material after your license expires is a violation of NRC regulations. A license will not normally be renewed, except on a case-by-case basis, in instances where licensed material has never been possessed or used.

In addition, please note that NRC Form 313 requires the applicant, by his/her signature, to verify that the applicant understands that all statements contained in the application are true and correct to the best of the applicant's knowledge. The signatory for the application should be the licensee or certifying official rather than a consultant.

You will be periodically inspected by NRC. Failure to conduct your program in accordance with NRC regulations, license conditions, and representations made in your license application and supplemental correspondence with NRC will result in enforcement action against you. This could include issuance of a notice of violation, or imposition of a civil penalty, or an order suspending, modifying or revoking your license as specified in the General Policy and Procedures for NRC Enforcement Actions. Since serious consequences

to employees and the public can result from failure to comply with NRC requirements, prompt and vigorous enforcement action will be taken when dealing with licensees who do not achieve the necessary meticulous attention to detail and the high standard of compliance which NRC expects of its licensees.

Information submitted in response to this letter should be referenced as ADDITIONAL INFORMATION TO PREVIOUS CONTROL NUMBER 97896.

Sincerely,

Original Signed By
Patricia J. Pelke
Nuclear Materials Licensing Branch

License No.: 48-26067-01
Docket No.: 030-31338

Enclosures:
1. Amendment No. 04 to License No. 48-26067-01
2. "Required Information for the Termination of Licensed Facilities"

DOCUMENT NAME: M:\03031338.CL6

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OFFICE	DNMS/RIII							
NAME	PPELKE:jaw							
DATE	12/ /96							

OFFICIAL RECORD COPY

Novagen

Novagen, Inc. 597 Science Dr. Madison, WI 53711 Telephone (608) 238-6110 Fax (608) 238-1388

Materials Licensing Section
U.S. Nuclear Regulatory Commission, Region III
801 Warrenville Road
Lisle, IL 60532-4351

September 20, 1996

Dear Reviewer,

We are submitting a change in our renewal application for our NRC license (#48-2607-01; Control number 97896). The original renewal application was dated November 28,1994.

1. Change of address: Novagen is moving to a new location at 601 Science Drive, Madison, WI on December 6, 1996. The new facililty is 18000 square feet of which 4700 square feet are devoted to laboratory space. We are isolating all radioisotope work to room number 155, which is 216 square feet. (See attached maps.) All of the radioisotope work, including inventory storage, waste storage, and experiments will be done in this one room. We have enclosed a new wipe test for room 155.

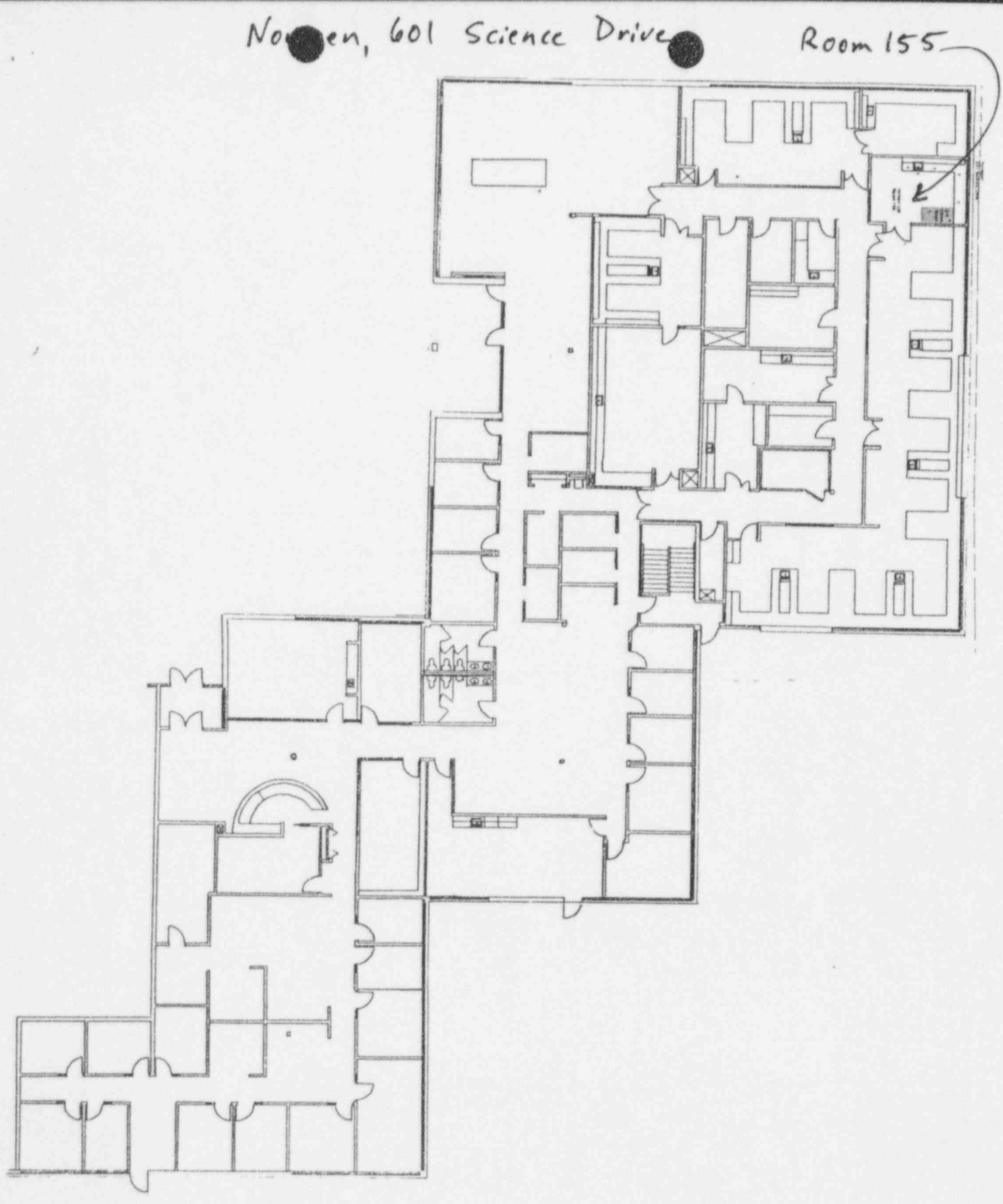
2. Deletion of address: We indicated in the renewal application on November 28,1994 that we would add the address at 595 Science Drive, Madison, WI. We have not and will not use this laboratory for radioisotope work, therefore we request to delete that location/laboratory from the license renewal. (No radioisotopes have been used at this address, therefore a close out survey will not be required at 595 Science Drive.)

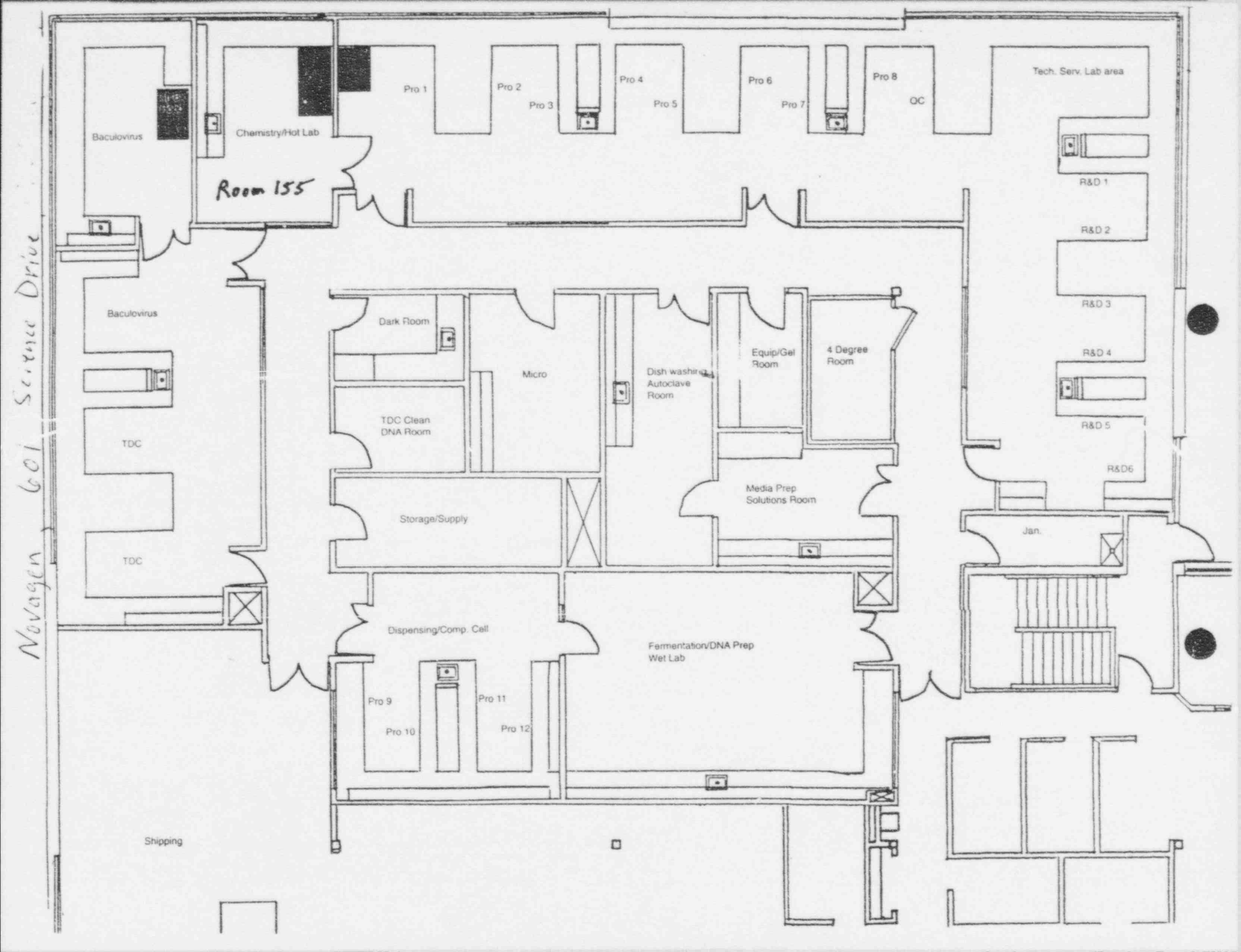
Please call me if I can be of further assistance.

Sincerely,

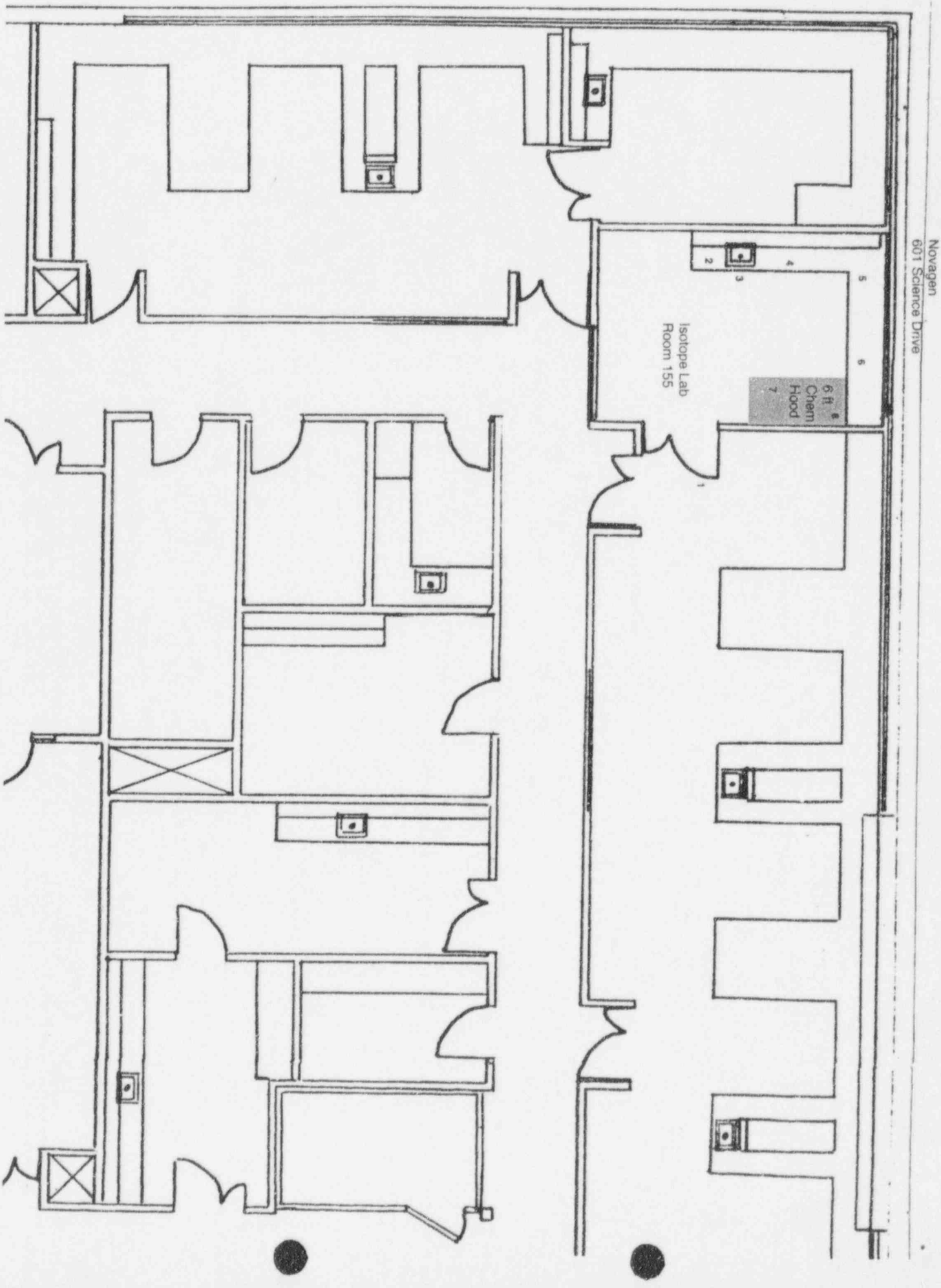
Robert C. Mierendorf
Vice President and General Manager

PM: 9-20-96


Nowen, 601 Science Drive
Room 155


Novagen 601 Science Drive
Baculovirus
Chemistry/Hot Lab
Room 155
Pro 1
Pro 2
Pro 3
Pro 4
Pro 5
Pro 6
Pro 7
Pro 8
QC
Tech. Serv. Lab area
R&D 1
R&D 2
R&D 3
R&D 4
R&D 5
R&D6
Baculovirus
TDC
TDC
Dark Room
Micro
TDC Clean
DNA Room
Storage/Supply
Dish washing
Autoclave
Room
Equip/Gel
Room
4 Degree
Room
Media Prep
Solutions Room
Jan.
Dispensing/Comp. Cell
Pro 9
Pro 10
Pro 11
Pro 12
Fermentation/DNA Prep
Wet Lab
Shipping

Novagen, 601 Science Drive



Wipe Test for Room 155

Surveyor's Name:_____

Date:_____

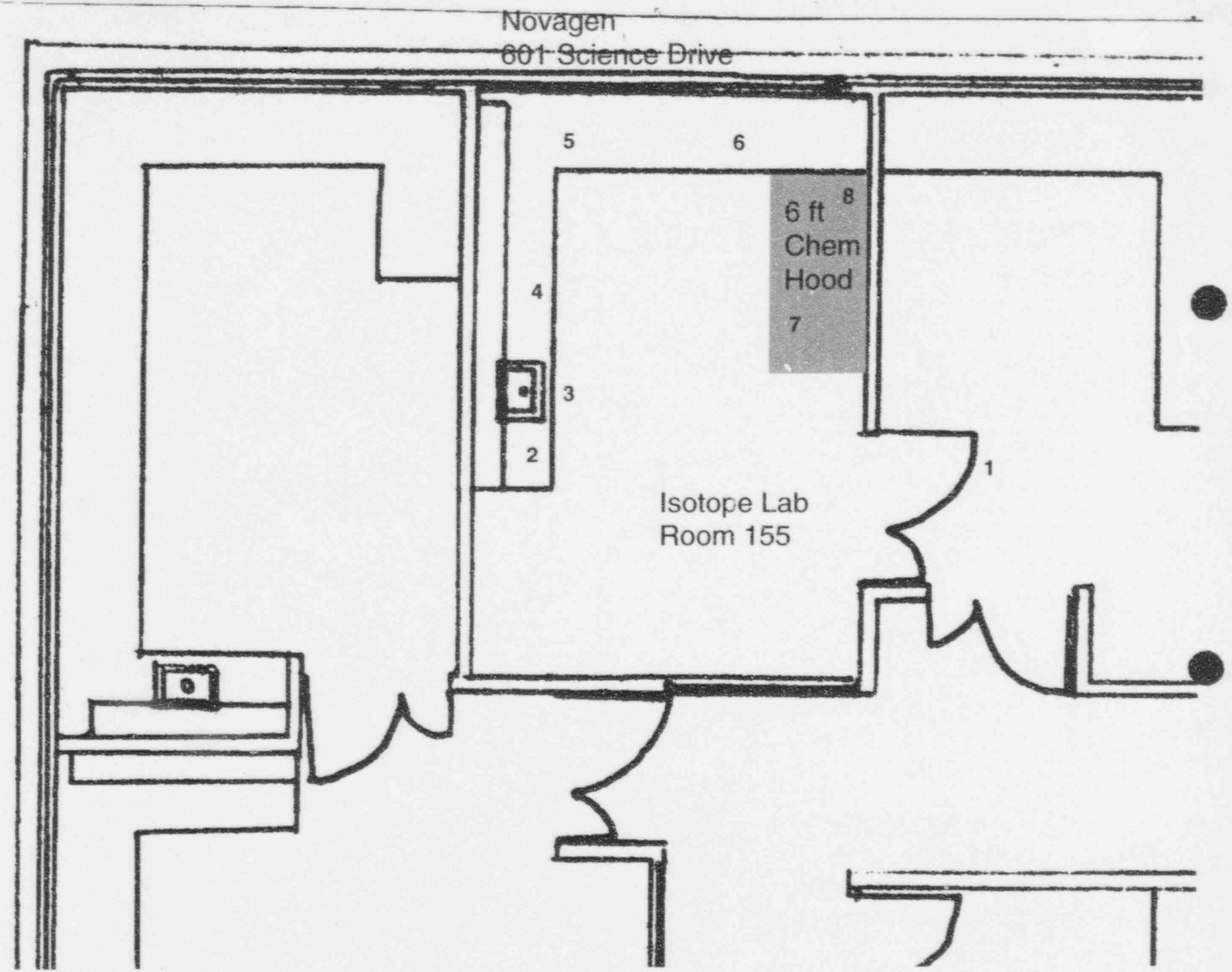
Area wiped (refer to map):

0. background sample (blank)
1. door to room 155
2. counter top
3. sink
4. counter top
5. counter top
6. counter top
7. counter top in the hood
8. counter top in the hood

Please attach cpm data.
Any area showing greater than 1000 cpm must be cleaned until wipe tests show levels of 1000 cpm or less.

Novagen, 601 Science Drive

Wipe Test Map for Room 155



CONVERSATION RECORD

TIME 9:45 am DATE 9/4/96

O VISIT O CONFERENCE X TELEPHONE

O INCOMING
X OUTGOING

NAME OF PERSON(S) CONTACTED OR IN CONTACT	ORGANIZATION (OFFICE, DEPT.ETC.)	TELEPHONE NO.
TOM VAN OOSBREE	NOVAGEN	608-238-6110

SUBJECT
LICENSE NO. 48-26067-01

SUMMARY
I CONTACTED T. VAN OOSBREE TO DISCUSS HIS INQUIRY REGARDING A NEW LOCATION OF USE FOR THE FACILITY. NOVAGEN WILL BE MOVING TO NEW FACILITIES IN DEC/JAN. I STATED THAT THEY SHOULD SEND US THE NEW ADDRESS AND A DIAGRAM OF THE NEW LOCATION AND WE WILL INCORPORATE THAT INTO THE RENEWAL. MR. VAN OOSBREE STATED THAT THE RENEWAL APPLICATION DATED 11/28/94 INCLUDED AN ADDITIONAL LAB (595 SCIENCE DRIVE) TO BE ADDED TO THE LICENSE. HE ALSO STATED THAT THEY NEVER OCCUPIED THIS LAB AND MATERIAL WAS NOT USED IN THAT LAB. I INDICATED THAT THEY SHOULD INCLUDE THIS INFORMATION ALONG WITH THE NEW FACILITY REQUEST. WE ALSO DISCUSSED THE CLOSE-OUT REQUIREMENTS FOR THE CURRENT FACILITY LOCATED AT 597 SCIENCE DRIVE.

LICENSEE WILL SUBMIT THIS INFORMATION BY 9/30/96 AND REFER TO CN 97896.

ACTION REQUIRED
ENTER MS 15 FOR CN 97896

NAME OF PERSON DOCUMENTING CONVERSATION	SIGNATURE	DATE
PATRICIA PELKE	P J Pelke	9/4/96

ACTION TAKEN

SIGNATURE TITLE DATE

December 9, 1994

Novagen
ATTN: Robert C. Mierendorf
Radiation Safety Officer
597 Science Drive
Madison, WI 53711

SUBJECT: LICENSE RENEWAL APPLICATION

Dear Mr. Mierendorf:

This is to acknowledge receipt of your application for renewal of the material(s) license identified above. Your application is deemed timely filed, and accordingly, the license will not expire until final action has been taken by this office.

Any correspondence regarding the renewal application should reference the control number specified and your license number.

Sincerely,

Original Signed By
Marianne Meenan, Chief
Nuclear Materials Support Section

License No. 48-26067-01
Control No. 97896

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OFFICE	DRSS/RIII									
NAME	MMEENAN:jaw									
DATE	12/ /94									

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