

UNIVERSIDAD DE PUERTO RICO
Recinto de Río Piedras
Facultad de Ciencias Naturales
Departamento de Biología
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UNIVERSITY OF PUERTO RICO
Río Piedras Campus
Faculty of Natural Sciences
Department of Biology
PO Box 23360
San Juan PR 00931-3360

April 3, 2006

Dennis R. Lawyer
Health Physicist
Commercial and R&D Branch
Division of Nuclear Materials Safety
US Nuclear Regulatory Commission
Region I
475 Allendale Road
King of Prussia, PA 19406-1415

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J-6
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RECEIVED
REGION 1

Re: Mail Control No. 138417

Dear Mr. Lawyer:

03001163

This is in reference to your letter of March 22, 2006, requesting more information about Dr. Irving Vega in order to continue to review our application for amendment of our license 52-01986-04. Enclosed you will find a letter from Dr. Vega giving more detail about his previous experience in handling radionuclides, which I hope will satisfy your request. The building and room where the work will be carried out, as also stated in his letter and in the layout of his lab, is Julio García Building, Room 120.

I am at your service should you require any further information,

Sincerely,

Fernando L. Renaud
Radiation Safety Officer

xc. Dr. Gladys Escalona de Motta, Chancellor

138417

NMGS/RGN MATERIALS-002

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University of Puerto Rico – Río Piedras
Faculty of Natural Sciences
Department of Biology
San Juan, Puerto Rico 00931

April 3, 2006

Dr. Fernando Renaud
Professor
Department of Biology
University of Puerto Rico – Río Piedras

Dear Dr. Renaud,

The present is to request authorization for the use of radioactive isotopes in my laboratory (JGD #120). The isotopes that my lab will employ are ^{32}P and ^{45}Ca . These isotopes will be used in *in vitro* experiments designed for the characterization of kinases and calcium-binding proteins. The protocols for radioactive assays are as follow:

^{45}Ca Calcium overlay assay: Purified recombinant proteins will be subjected to native or SDS-PAGE electrophoresis. The resolved proteins will be transferred to nitrocellulose. After transfer, the membrane is soaked in equilibration buffer [10mM HEPES (pH7.4), 60mM KCl, 5mM MgCl_2] for 1hr with three buffer changes. Then, the membrane is going to be incubated in equilibration buffer containing $1\mu\text{Ci/mL}$ ^{45}Ca for 30min at room temperature. The membrane is washed three times with distilled water (5min/each) at room temperature (RT). The membrane is air dried and exposed to X-ray film for various time intervals.

^{45}Ca Calcium binding assay: Purified recombinant proteins will be purified and equilibrated in binding buffer [10mM Tris-HCl (pH 7.5), 100mM KCl]. The beads containing the purified proteins will be incubated in binding buffer containing $1\mu\text{Ci/mL}$ ^{45}Ca with or without 20mM EDTA for 30min at RT. The supernatant will be removed and the beads washed three times with binding buffer. The radioactivity associated to the bound proteins would be measured by liquid scintillation counting.

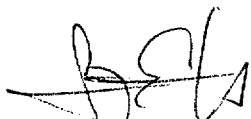
Phosphorylation assay: Immunopurified proteins will be equilibrated in kinase buffer [20mM HEPES (pH7.5), 10% glycerol, 5mM MgCl_2 , 10mM MnCl_2]. Specific kinases will be added and incubated with the immunopurified substrate in presence of $1\mu\text{Ci}$ [γ - ^{32}P]ATP, for 20min at RT. The beads containing the immunopurified protein will be washed several times. Sample loading buffer [60mM Tris-HCl (pH6.8), 2%SDS, 10% glycerol, 0.025% Bromophenol Blue, 5% 2-mercaptoethanol] will be added to stop the reaction. The proteins are resolved in SDS-PAGE. The gel will be dried and exposed to X-ray film.

In my career, I have received training on and worked with radioactive isotopes [e.g. Schaubert, C. et al., (1998) *Nature* 391:715-718; Schaubert, C. et al., (1998) *Genes to Cell* 3:307-319]. The radioactive isotopes previously used are $\gamma^{32}\text{P}$ -ATP, Orthophosphate- ^{32}P , Methionine- ^{35}S and Cysteine- ^{35}S . The specific amount used varies from experiment to experiment, being

Orthophosphate- $i^{32}\text{P}$ the most used with $\sim 1\mu\text{Ci}$ per experiment. These prior experiences allow me to understand the management, security and responsibilities associated to the use of radioactive isotopes, specifically those emitting beta-particle radiation. This radioactive work was carried out from August 1996 to January 2002. Please see attached a letter from Rutgers University's Environmental Health and Safety office stating the dates from which I received training.

Your consideration and support in this regard will have a direct impact on my research goals.

Sincerely,



Dr. Irving E. Vega
Assistant Professor
Department of Biology
University of Puerto Rico
Julio García Díaz #120
San Juan, Puerto Rico 00931

THE STATE UNIVERSITY OF NEW JERSEY

RUTGERS

Rutgers Environmental Health and Safety
Rutgers, The State University of New Jersey
27 Road I • Piscataway • New Jersey, 08854-8036
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February 2, 2006

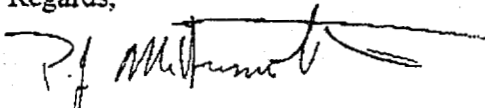
To Whom It May Concern:

This is to certify that Dr. Irving Vega attended an Initial Radiation Safety Training session presented by Rutgers Environmental Health and Safety in 1996. Subsequently, Dr. Vega attended refresher radiation safety training sessions in 1997, 1999, 2001 and 2002.

Radiation Safety Training is designed to instruct the participants how to handle and work safely with radioactive materials at Rutgers University and the Robert Wood Johnson Medical School (RWJMS). Topics covered include, but are not limited to: biological effects of ionizing radiation, dose and dose rates, instrumentation and monitoring techniques, safe handling techniques, ALARA, contamination control and emergency response procedures. Be advised that some of the training material, most notably emergency procedures (whom and when to contact) and waste disposal, are specific to Rutgers University.

Please feel free to contact me if you have questions regarding the content of our Radiation Safety Training.

Regards,



Patrick McDermott
University Health Physicist
mcdermot@rehs.rutgers.edu

Dr. Vega's Laboratory
Julio Garcia Diaz Building #120

Radioactive Material Work - Layout

