NRC FORM 374
(7-94)

U.S. NUCLEAR REGULATORY COMMISSION

MATERIALS LICENSE

Pursuant to the Atomic Energy Act of 1954, as amended, the Energy Reorganization Act of 1974 (Public Law 93-438), and Title 10 Federal Regulations, Chapter I, Parts 30, 31, 32, 33, 34, 35, 36, 39, 40, and 70, and in reliance on statements and representations heretofore 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.

OFFICIAL RECORD COPY

Licensee	
1. CIS-US, Inc.	3. License Number 20-20973-05G
2. 10 DeAngelo Drive Bedford, Massachusetts 01730	4. Expiration Date March 31, 2007
	5. Docket or Reference No. 030-34394

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. Iodine 125	A. Prepackaged units	A. Not applicable

9. Authorized use

A. Pursuant to 10 CFR 32.71, the licensee is authorized to distribute in vitro kits containing prepackaged units to persons generally licensed pursuant to 10 CFR 31.11, or equivalent provisions of the regulations of any Agreement State.

CONDITIONS

10. The licensee may distribute material from 10 DeAngelo Drive, Bedford, Massachusetts.

11. The licensee may not possess and use materials authorized in Items 6, 7, and 8, until: (1) the licensee has constructed the facilities and obtained the equipment described in the application and supporting documentation; and (2) the U.S. Nuclear Regulatory Commission, Region I, ATTN: Chief, Nuclear Materials Safety Branch, 475 Allendale Road, King of Prussia, Pennsylvania 19406 has been notified in writing that activities authorized by the license will be initiated.

In accordance with the requirements set forth in 10 CFR 30.36(b), 40.42(b), and 70.38(b), the licensee shall promptly notify the Nuclear Regulatory Commission, in writing, of a decision not to complete the facility, acquire equipment, or possess and use authorized material.

12. This license does not authorize possession or use of licensed material.

150023

ML 10

MATERIALS LICENSE
SUPPLEMENTARY SHEET

License Number: 20-20973-05G

Docket or Reference Number: 030-34394

13. 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 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 February 7, 1997
B. Letter February 12, 1997

For the U.S. Nuclear Regulatory Commission

Date MAR - 6 1997

By Original Signed By:
John R. McGrath
Division of Nuclear Materials Safety
Region I
King of Prussia, Pennsylvania 19406

MAR - 6 1997

License No. 20-20973-05G
Docket No. 030-34394
Control No. 124255

Mr. David B. Reader
Vice President
CIS-US, Inc.
10 DeAngelo Drive
Bedford, MA 01730

Dear Mr. Reader:

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 I Office, Licensing Assistance Team, (610) 337-5093 or 5239, so that we can provide appropriate corrections and answers.

Please be advised that your license expires at the end of the day, in the month, and year stated in the license. Until your license is 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. Not possess and use materials authorized in Items 6, 7, and 8, on the license until:

   a. you have constructed the facilities and obtained the equipment described in the license application and supporting documentation; and

   b. you have notified the U.S. Nuclear Regulatory Commission, Region I, ATTN: Chief, Nuclear Materials Safety Branch, 475 Allendale Road, King of Prussia, Pennsylvania 19406 in writing, that activities authorized by the license will be initiated.

3. Notify NRC, in writing, within 30 days when the mailing address on the license changes (no fee is required if the location of byproduct material remains the same).

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4. In accordance with 10 CFR 30.36(b) and/or license condition, notify NRC, promptly, in writing, and request termination of the license:

   a. when you decide to terminate all activities involving materials authorized under the license; or

   b. if you decide not to complete the facility, acquire equipment, or possess and use authorized material.

5. Request and obtain a license amendment before you:

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

   b. add or change the areas of use, or address or addresses of use identified in the license application or on the license; or

   c. change ownership of your organization.

6. 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 a certifying official of the licensee rather than the Radiation Safety Officer or a consultant.

You will be periodically inspected by the 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 Statement of Policy and Procedure for NRC Enforcement Actions," (Enforcement Policy), NUREG 1600.

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.

Thank you for your cooperation.

Sincerely,

Original Signed By:

John R. McGrath
Senior Health Physicist
Division of Nuclear Materials Safety

License No. 20-20973-05G
Docket No. 030-34394
Control No. 124255

Enclosures:
1. License No. 20-20973-05G
2. 10 CFR Parts 2, 19, 20, 30, 31, 32 and 170
3. NRC Form 3 and 313

DOCUMENT NAME: R:\WPS\MLTR\L2020973.05G
To receive a copy of this document, indicate in the box: "C" = Copy w/o attach/encl "E" = Copy w/ attach/encl "N" = No copy

OFFICE	DNMS/RI	N	DNMS/RI					
NAME	McGrath JAM							
DATE	02/12/97		02/ /97		02/ /97		02/ /97	

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CIS-US, Inc.

Subsidiary of CIS bio International

February 12, 1997

030-34394

U.S. Nuclear Regulatory Commission, Region I
Mr. John R. McGrath
Nuclear Materials Safety Branch
475 Allendale Road
King of Prussia, PA 19406

Dear Mr. McGrath:

Further to our telephone conversation today regarding our application to distribute the ELSA-OSTEO RIA Kit for *in vitro* testing under general license:

We enclose revised page nos. 2 and 3, effective 2/12/97, to replace those of the original application, dated February 7, 1997. I believe that the label exhibits on page 3 will satisfy the requirements that you indicated.

Thank you very much for your prompt action and timely issuance of this license. Please contact me immediately for any action on our part which will help further that crucial objective.

Sincerely,

Paul M. Tyree
Radiation Safety Officer

encl: Applicationpages noted above (2 copies)

cis-us, inc. • 10 de angelo drive • bedford, ma 01730 • tel. (617) 275-7120 • fax (617) 275-2634

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NRC FORM 313 ITEMS 7, 8, 9 AND 11 NOT APPLICABLE FOR THIS LICENSE.

5. RADIOACTIVE MATERIAL

a. Material	b. Chemical and/or physical form	c. Maximum amount
(1) Iodine-125	(1) ELSA-OSTEO *in vitro* RIA Kit	(1) **8 μCi [296 kBq]** labeled activity at manufacture. (Rev. 2/12/97)

6. USE OF RADIOACTIVE MATERIAL

Item 5 a.-c.(1) Distributi · by CIS-US pursuant to 10 CFR 32.71; to persons generally licensed under § 31.11 or equivalent general licensure of an Agreement State.

This license will not authorize possession or use of licensed material.[See 20-20973-01]

10. RADIATION SAFETY PROGRAM

Item 5 a.-c.(1)

1. The ELSA-OSTEO *in vitro* RIA Kit will be manufactured in France by CIS bio international (CBI), and supplied prepacked to CIS-US with package, labeling and instruction booklet as exhibited in **Attachment A**, page 3.

2. Transfer of kits to generally licensed persons will be subject to verification of recipient's registration or license pursuant to § 31.11(b) or equivalent general licensure of an Agreement State, in compliance with the requirements and methods specified in § 30.41(c) and (d).

CIS-US, Inc.
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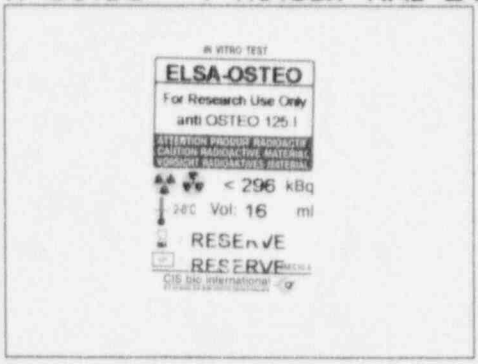
ATTACHMENT A

Exhibit: ELSA-OSTEO RIA KIT (Rev. 2/121/97)

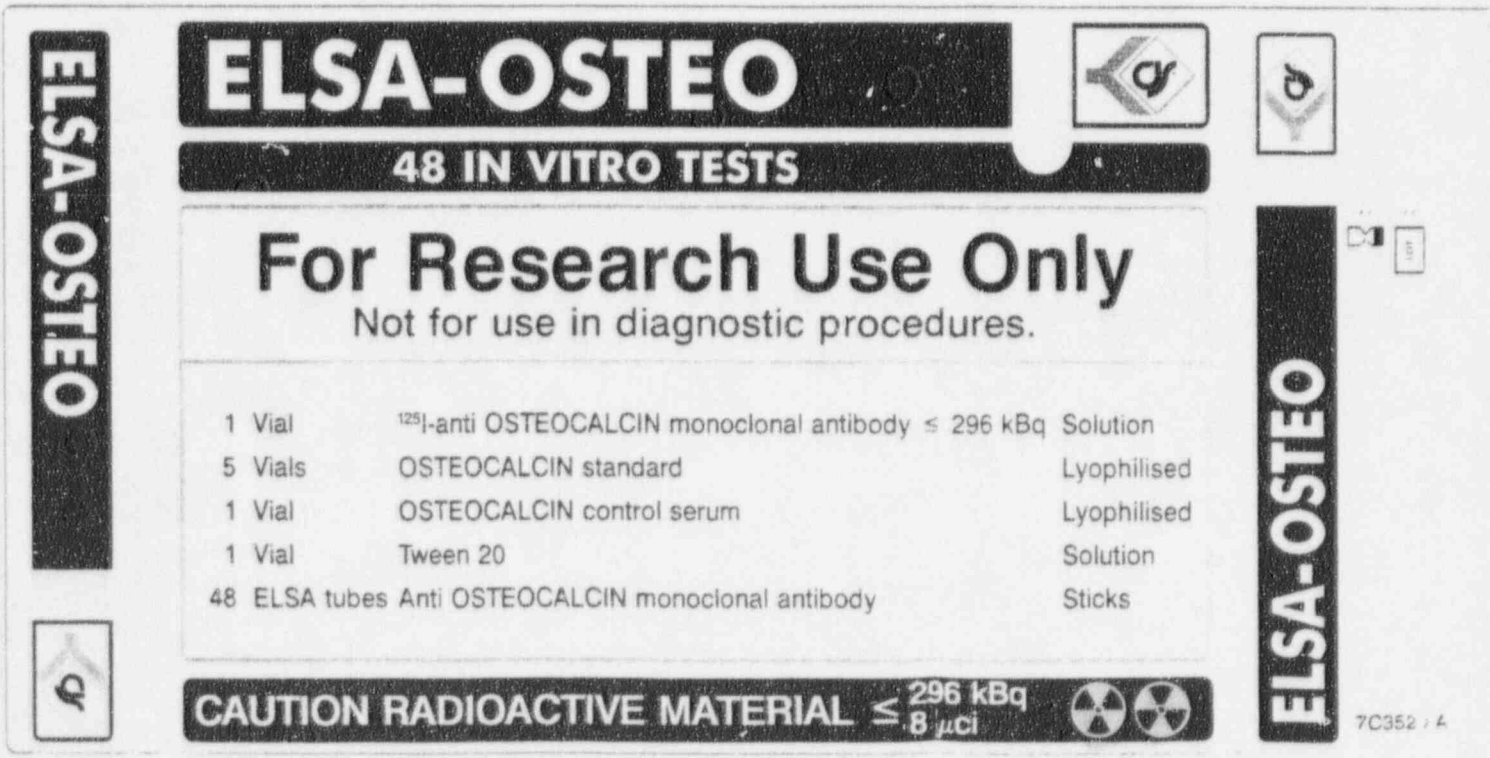
Side 1

Side 2 Attachment: Package Insert Graphic

ANTI OSTEO ^{125}I TRACER VIAL LABEL



KIT PACKAGE LABEL (65 % ACTUAL SIZE)



ELSA-OSTEO IMMUNORADIOMETRIC ASSAY

PACKAGE INSERT

For Research Use Only
Not for Diagnosis

VERSION **ANGLAISE**

Réf. : ELSA-OSTEO

Mise à jour le 03/02/97

(48 tubes)

Février 1997 - Modèle US 01

For Research Use Only - Not for use in diagnostic procedures

The radioactive material in this *in vitro* RIA kit may be received, acquired, possessed and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

Distributed by CIS-US, Inc., 10 DeAngelo Dr., Bedford, MA 01730

1. NAME

ELSA-OSTEO is a kit for the immunoradiometric assay of osteocalcin (Bone GLA Protein) in human serum and plasma.

2. INTRODUCTION

Osteocalcin or Bone GLA Protein (BGP) is a low molecular weight protein (5800 D). Synthesized by osteoblasts, it is specific to bone tissue and represents approximately 20 % of non-collagenous proteins. This 49 amino acids peptide contains 3 gamma-carboxy-glutamic acide (GLA) and has 2 potential sites for tryptic hydrolysis at the 19-20 and 43-44 residue levels. The resulting 1-19, 20-43, 44-49, 1-43 et 20-49 peptides may be the products of breakdown in the liver, kidney or plasma.

3. PRINCIPLE

ELSA-OSTEO is a solid-phase «sandwich» immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites. The first is coated on the ELSA solid phase ; the second, radiolabeled with iodine 125, is used as a tracer

Osteocalcin molecules present in the standards or the samples to be tested are «sandwiched» between the two antibodies. Excess unbound tracer is easily removed during the procedure's washing step, and the ELSA retains only the adsorbed antibody/antigen/tracer antibody combination.

The amount of radioactivity bound to the ELSA is proportional to the amount of osteocalcin present at the beginning of the assay.

4. REAGENTS

Each kit contains enough reagents for 48 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
ELSA : ready for use Monoclonal anti-human osteocalcin antibody coated on ELSA fixed to the bottom of the tube.	2 traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
ANTI OSTEO ^{125}I : ready for use ^{125}I monoclonal anti-human osteocalcin antibody, buffer, animal proteins, sodium azide, red dye, non-immunized mice immunoglobulins. ≤ 296 kBq (≤ 8 µCi)	1 16 ml vial	2-8°C until the expiry date.
STANDARD 0 : lyophilized Buffer, animal proteins. Reconstitute the vial's content with 4 ml of distilled water.	1 qs 4 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
STANDARDS : lyophilized Buffer, animal proteins, human osteocalcin 5 - 40 - 120 - 300 ng/ml* Reconstitute the vial's content with 0.5 ml of distilled water.	4 qs 0.5 ml vials	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C .**
CONTROL : lyophilized Buffer, animal proteins, human osteocalcin 20 ng/ml* Reconstitute the vial's content with 0.5 ml of distilled water.	1 qs 0.5 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
TWEEN 20 : concentrated solution Dilute 9 ml of Tween 20 in 3 liters of distilled water. Shake gently.	1 10 ml vial	2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.
PLASTIC BAG	1	

(*) The values shown above are only target values : the true value of each standard or control is shown on its label.

(**) Standards and control should be frozen and thawed once.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Human tissue derivatives contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all human tissue derived products including the samples to be assayed must be treated as potentially infectious.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

The radioactive material may be received, acquired, possessed and used only by physicians, veterinarians in the pratice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement of the exercise of regulatory authority.

Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone.
Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water used for washing.
Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma, haemolyzed or hyperlipemic samples should not be used. Heparin or EDTA plasma samples should be used. Do not use citrate as an anticoagulant. If the test is to be carried out within 4 hours, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed.

Dilutions
Should elevated osteocalcin levels be suspected, the O Standard found in the kit is used for dilution.
It is recommended to carry out the dilutions using disposable plastic tubes.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 50 µl, 300 µl, 500 µl and 4 ml (± 1%). Their calibration should be checked regularly.
Distilled water.
Disposable plastic tubes.
Vortex-type mixer.
Circular horizontal shaker.
Gamma scintillation counter calibrated for 125 iodine measurement.

Equipment suitable for this assay is available from CIS bio international ; information on request.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Standards and control must be reconstituted 15 minutes before use. Dispensing of the reagent into the ELSA tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes :

O Standard group, for the determination of non-specific binding.
Standard groups, to establish the standard curve.
Control group for the control.
Sx groups, for the test samples.
It is recommended that the assay be performed in triplicate for the standard and in duplicate for the samples.

Strictly observe the order in which reagents are to be added :

Dispense 50 µl of standards, or control or research samples to be assayed, into the corresponding-labeled ELSA tubes.
Add 300 µl of ^{125}I anti-human osteocalcin to each ELSA tube.
Mix each tube gently with a Vortex-type mixer.
Incubate for 2 h ± 5 mn at room temperature (18-25°C) under agitation.

Wash the ELSA tubes as follows :

Aspirate the contents of the tubes as completely as possible.
Add 3.0 ml of washing solution to each tube, and re-empty. Repeat this process twice.
To obtain reliable and reproducible results, the different washing steps have to be correctly performed. As much as possible of the incubation and washing solutions must be removed. If manual aspiration is used, the tip of the aspirating device must be placed right at the bottom of the tube.

Measure the remaining radioactivity bound to the ELSA with a gamma scintillation counter.

8. QUALITY CONTROL

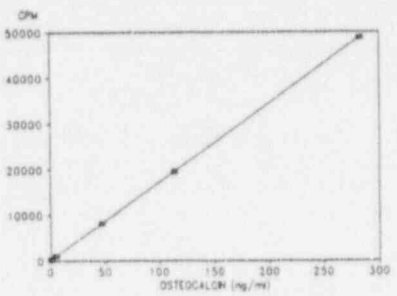
This kit is intended for Research use only and not for any specific application.
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.
Draw up the standard curve by plotting the standard's cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

Typical standard curve (example only) : these data must not be substituted for results obtained in the laboratory.

Tube Groups	Mean cpm	Concentration ng/ml
Standard O	205	0
Standard 1	1 064	5
Standard 2	8 094	47
Standard 3	19 532	114
Standard 4	48 735	283
Control	3 510	20
Sample 1	12 105	72
Sample 2	29 090	171



10. PROCEDURAL LIMITATIONS

Research samples containing fibrin, gross haemolysis, gross lipemia or turbidity may give inaccurate results.
Do not attempt to extrapolate sample values beyond the last standard. Dilute the samples and retest.

11. EXPECTED VALUES (example only)

The values below are only given as an indication and it is recommended that each laboratory establish its own range of normal values.
The chart below shows the distribution of values obtained in presumably normal adult subjects.

	Age (years)	Number of cases	Mean ng/ml	Median ng/ml	Extreme values ng/ml
Males	20 - 30	48	23.8	22.0	11.3 - 37.0
	31 - 40	51	21.5	19.4	10.7 - 34.1
	41 - 50	49	20.3	19.9	5.2 - 34.5
	51 - 60	91	18.7	18.6	6.3 - 30.7
	61 - 70	60	19.1	19.2	8.8 - 29.7
Females	20 - 30	70	21.8	21.1	8.8 - 39.4
	31 - 40	87	17.1	16.2	7.7 - 31.9
	41 - 50	74	15.7	15.0	8.0 - 36.0
	51 - 60	85	24.4	22.6	8.0 - 50.5
	61 - 70	32	24.4	24.3	12.9 - 55.9

The values have to take into account age and sex.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY (example only)

12.1. Imprecision

This has been assessed using 2 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 20 different series.

Sample	Mean ng/ml	Within-run CV %	Between-run CV %
1	21.9	3.8	5.2
2	183.9	3.9	4.5

12.2. Recovery test

Known quantities of human osteocalcin were added to human sera. The recovery percentages of human osteocalcin in the samples ranged from 95 to 105 %.

12.3. Dilution test

Ten samples with high levels were diluted, with the recovery percentages ranging from 95 to 105 %.

12.4. Specificity

ELSA-OSTEO measures the 1-49 human osteocalcin (carboxylated or decarboxylated) and human osteocalcin peptide 1-43.
On the other hand, human osteocalcin peptides 7-19, 25-37 and 37-49 do not interfere in the ELSA-OSTEO assay for concentration < 10 000 ng/ml.

12.5. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.4 ng/ml.

This kit is intended for Research Use only and is not intended to be used for patient diagnosis or follow-up.

ASSAY FLOW-CHART

Tubes	Standards Control Samples µl	^{125}I anti-human osteocalcin µl	Mix Incubate 2 H ± 5 mn à 18-25°C under agitation Wash 3 times with the washing solution	Count
Standards	50	300		
Control or Samples	50	300		

REFERENCES

Delmas PD, Christiansen C, Mann KG, Price PA. Bone Gla-protein (osteocalcin) assay standardization report. J Bone Min Res. 5(1): 5-11, 1990.

Hauschka PV, Lian JE, Gallop PM. Direct identification of the calcium-binding amino acidgamma-carboxyglutamate in mineralized tissue. Proc Nat Acad Sci USA. 72: 3925-29, 1975.

Price PA, Ostuka AS, Poser JW et al. Characterization of gamma-carboxyglutarnic acid-containing protein from bone. Proc Nat Acad Sci USA. 73: 1447-51, 1976.

Vermeulen AHM, Vermeer C, Bosman FT. Histochemical detection of osteocalcin in normal and pathological human bone. J Histochem Cytochem. 37 (10): 1503-08, 1989.

Octobre 1994 - Modèle 05

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For Research Use Only - Not for use in diagnostic procedures

The radioactive material in this *in vitro* RIA kit may be received, acquired, possessed and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

Distributed by CIS-US, Inc., 10 DeAngelo Dr., Bedford, MA 01730

ELSA-OSTEO

CIS bio international
ORIS Group
B.P. 32 - F91192 GIF-SUR-YVETTE CEDEX / FRANCE
Tél. FRANCE (33) 1-69.85.72.80 - Fax 33 (1) 69.85.73.65 - Télex 603912

1. NAME AND INTENDED USE

ELSA-OSTEO is a kit for the immunoradiometric assay of osteocalcin (Bone GLA Protein) in human serum and plasma.

2. INTRODUCTION

Osteocalcin or Bone GLA Protein (BGP) is a low molecular weight protein (5800 D). Synthesized by osteoblasts, it is specific to bone tissue and represents approximately 20 % of non-collagenous proteins. This 49 amino acids peptide contains 3 gamma-carboxy-glutamic acide (GLA) and has 2 potential sites for tryptic hydrolysis at the 19-20 and 43-44 residue levels. The resulting 1-19, 20-43, 44-49, 1-43 et 20-49 peptides may be the products of liver, kidney and plasmatic breakdown of the molecule. The physiological role of these fragments is unknown.

Physiologically, the osteocalcin level is elevated in children, particularly during the first year of life and during puberty when the evolution of the level is related to the rapidity of physical growth. For adults, the circulating osteocalcin level must be interpreted according to the age and sex of the patient.

In adults, there is a correlation between osteocalcin and the rate of bone turn over. Its presence is more marked in cases of hyper-remodeling (renal osteodystrophy, early hyperparathyroidism, hyperthyroidism, Paget's disease) and reduced in cases of hypo-remodeling (hypoparathyroidism, hypercalcemia resulting from bone metastasis and long term cortisone therapy).
When the processes of bone resorption and formation are out of phase (cortisone therapy, osteoporosis) serum osteocalcin provides a specific indicator of bone formation - i. e. of osteoblastic activity within the skeleton as a whole - and does not reflect bone resorption.

In primary osteoporosis, such as in the post-menopausal form when the phosphocalcic balance is normal, the assay of osteocalcin levels allows both classification of the disease according to regeneration rate (osteoporosis with high remodeling, osteoporosis with low remodeling) and a systematic follow-up of the patients treated.

During prolonged cortisone therapy, the osteocalcin measurement allows an evaluation of patient's osteoblastic activity. Here, a decline in osteoblastic activity is accompanied by a decrease of the osteocalcin level, with the decrease of osteocalcin level being in proportion to the amount of cortisone prescribed.

3. PRINCIPLE

ELSA-OSTEO is a solid-phase "sandwich" immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites. The first being coated on the ELSA solid phase ; the second, radiolabeled with iodine 125, is used as a tracer.

Ostnocalcin molecules present in the standards or the samples to be tested are "sandwiched" between the two antibodies. Excess unbound tracer is easily removed during the procedure's washing step, and the ELSA retains only the adsorbed antibody/antigen/tracer antibody combination.

The amount of radioactivity bound to the ELSA is proportional to the amount of osteocalcin present at the beginning of the assay.

4. REAGENTS

Each kit contains enough reagents for 48 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
ELSA : ready for use. Monoclonal anti-human osteocalcin antibody coated on ELSA fixed to the bottom of the tube.	traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
ANTI OSTEO ^{125}I : ready for use. ^{125}I monoclonal anti-human osteocalcin antibody, buffer, animal proteins, sodium azide, red dye, non-immunized mice immunoglobulins. ≤ 278 kBq (≤ 7.5 µCi)	1 x 15 ml Tracer	2-8°C until the expiry date.
STANDARD 0 : lyophilized. Buffer, animal proteins. Reconstitute with 4 ml of distilled water.	1 qs 4 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
STANDARDS : lyophilized. Buffer, animal proteins, human osteocalcin. 5 - 40 - 120 - 300 ng/ml*. Reconstitute with 0.5 ml of distilled water.	4 qs 0.5 ml vials	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
CONTROL : lyophilized. Buffer, animal proteins, human osteocalcin. 20 ng/ml*. Reconstitute with 0.5 ml of distilled water.	1 qs 0.5 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
TWEEN 20 : concentrated solution. Dilute 9 ml of Tween 20 in 3 liters of distilled water. Shake gently.	1 10 ml vial	2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.
PLASTIC BAG	1	

(*) The values shown above are only target values : the true value of each standard or control is shown on its label.

(**) Standards and control should be frozen and thawed once.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Human tissue derivatives contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all human tissue derived products, including the samples to be assayed, must be treated as potentially infectious.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3 Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water used for washing.
Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma, haemolyzed or hyperlipemic samples should not be used. Heparin or EDTA plasma samples should be used, citrate as anticoagulant have to be discarded. If the test is to be carried out within 4 hours, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed.

Dilution

Should elevated osteocalcin levels be suspected, the O Standard found in the kit is used for dilution.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 50 µl, 300 µl, 500 µl and 4 ml (± 1%). Their calibration should be checked regularly.
Distilled water.
Disposable plastic tubes.
Vortex-type mixer.
Circular horizontal shaker.
Gamma scintillation counter calibrated for 125 iodine measurement.

Equipment suitable for this assay is available from CIS bio international ; information on request.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Standards and control must be reconstituted 15 minutes before use. Dispensing of the reagent into the ELSA tubes is also carried out at room temperature (18-25°C).

The assay requires the following groups of tubes :
O Standard group, for the determination of non-specific binding.
Standard groups, to establish the standard curve.
Control group for the control.
Sx groups, for the samples to be assayed.
It is recommended to performe the assay in triplicate for the standards and in duplicate for the samples.

Observe the order in which reagents are to be added :

Dispense 50 µl of standards, or control or samples to be assayed, into the corresponding-labeled ELSA tubes.
Add 300 µl of ^{125}I anti-human osteocalcin to each ELSA tube.
Gently mix each tube with a Vortex-type mixer.
Incubate for 2 h (± 5 mn) at room temperature (18-25°C) under agitation.

Wash the ELSA tubes as follows :

Aspirate the content of the tubes as completely as possible.
Add 3.0 ml of washing solution to each tube, and re-empty.
Repeat the process twice.
To obtain reliable and reproducible results, the different washing steps have to be correctly performed.
As much as possible of the incubation and washing solutions must be removed. If manual aspiration is used, the tip of the aspirating device must be placed right at the bottom of the tube.
Measure the radioactivity bound to the ELSA with a gamma scintillation counter.

8. QUALITY CONTROL

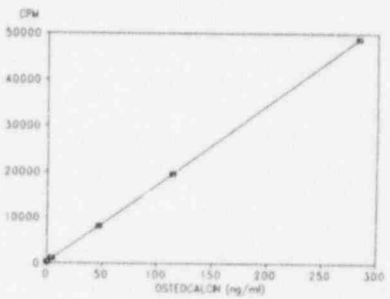
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.
Draw up the standard curve by plotting the standard's cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

Typical standard curve (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

Tube groups	Mean cpm	Concentration ng/ml
Standard 0	205	0
Standard 1	1 064	5
Standard 2	8 094	47
Standard 3	19 532	114
Standard 4	48 735	283
Control	3 510	20
Sample 1	12 105	72
Sample 2	29 090	171



10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results. Do not attempt to extrapolate sample values beyond the last standard. Dilute the samples concerned and retest.

11. EXPECTED VALUES

Each laboratory should establish its own range of normal values. Values shown below are only an example. The chart below shows the distribution of values obtained in presumably normal adult subjects.

	Age (years)	Number of cases	Mean ng/ml	Median ng/ml	Extreme values ng/ml
Males	20 - 30	48	23.8	22.0	11.3 - 37.0
	31 - 40	51	21.5	19.4	10.7 - 34.1
	41 - 50	49	20.3	19.9	5.2 - 34.5
	51 - 60	91	18.7	18.6	6.3 - 30.7
	61 - 70	60	19.1	19.2	8.8 - 29.7
Females	20 - 30	70	21.8	21.1	8.8 - 39.4
	31 - 40	87	17.1	16.2	7.7 - 31.9
	41 - 50	74	15.7	15.0	8.0 - 36.0
	51 - 60	85	24.4	22.6	8.0 - 50.5
	61 - 70	32	24.4	24.3	12.9 - 55.9

The values have to take into account age and sex.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 2 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 20 different series.

Sample	Mean ng/ml	Within-run CV %	Between-run CV %
1	21.9	3.8	5.2
2	183.9	3.9	4.5

12.2. Recovery test

Known quantities of human osteocalcin were added to human sera. The recovery percentages of human osteocalcin in the samples ranged from 95 to 105 %.

12.3. Dilution test

Ten samples with high levels were diluted with the recovery percentages ranging from 95 to 105 %.

12.4. Specificity

ELSA-OSTEO measures the 1-49 human osteocalcin (carboxylated or decarboxylated) and human osteocalcin peptide 1-43.
On the other-hand, human osteocalcin peptides 7-19, 25-37 and 37-49 do not interfere in the ELSA-OSTEO assay for concentration < 10 000 ng/ml.

12.5. Detection Limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.4 ng/ml.

ASSAY FLOW-CHART

Tubes	Standards Control Samples µl	^{125}I anti-human osteocalcin µl	Mix Incubate 2 H ± 5 mn à 18-25°C under agitation Wash 3 times with the washing solution	Count
Standards	50	300		
Control or Samples	50	300		

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Octobre 1994 - Modèle 05

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For Research Use Only - Not for use in diagnostic procedures

The radioactive material in this *in vitro* RIA kit may be received, acquired, possessed and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

Distributed by CIS-US, Inc., 10 DeAngelo Dr., Bedford, MA 01730

ELSA-OSTEO

CIS bio international

ORIS Group
B.P. 32 - F91192 GIF-SUR-YVETTE CEDEX / FRANCE
Tel. FRANCE (33) 1-69.85.72.60 - Fax 33 (1) 69.85.73.65 - Télex 603912

CIS-US, Inc.

Subsidiary of CIS bio international

February 7, 1997

L 20973
030-34394
03244

U.S. Nuclear Regulatory Commission, Region I
Att: Licensing Assistance Section
475 Allendale Road
King of Prussia, PA 19406

Sb: **Application for expedited review;** specific license to distribute pursuant to 10 CFR 32.71

Dear Sir/Madam:

We enclose a form 313 application to distribute licensed material for *in vitro* testing under general license, and remittance of a category 3.K license application fee in the amount of one thousand, three hundred dollars ($1300.00). It will be necessary to commence distribution of the ELSA-OSTEO RIA Kit for use under §31.11 or equivalent general licensure prior to the expected effective date of the pending agreement with Massachusetts. **NRC licensing of such prior to your cessation of actions on Massachusetts agreement materials licenses is urgently needed and will be greatly appreciated.**

Thank you very much for your prompt action and timely issuance of this license. Please contact us immediately for any action on our part which will help further that crucial objective.

Sincerely,

David B. Reader
Executive Vice President

encl: Application (2 copies)
Fee remittance, 53-235/113 , check 019670

124255

FEB 12 1997

FEB 11 1997 -

FAX REC'D _____

cis-us, inc. • 10 de angelo drive • bedford, ma 01730 • tel. (617) 275-7120 • fax (617) 275-2634

NRC FORM 313 (6-93)
10 CFR 30, 32, 33, 34, 35, 36, 39 and 40

U. S. NUCLEAR REGULATORY COMMISSION

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
799 ROOSEVELT ROAD
GLEN ELLYN, IL 60137-5927

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

L 20973
030-34394
03244

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)*

- [x] A. NEW LICENSE
- [ ] B. AMENDMENT TO LICENSE NUMBER _____
- [ ] C. RENEWAL OF LICENSE NUMBER _____

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

CIS-US, Inc.
10 DeAngelo Drive
Bedford, MA 01730

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

CIS-US, Inc.
10 DeAngelo Drive
Bedford, Masachusetts 01730

4 NAME OF PERSON TO BE CONTACTED ABOUT THIS APPLICATION

Paul M. Tyree

TELEPHONE NUMBER

(617) 275-7120

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	LICENSEE FEES *(See 10 CFR 170 and Section 170.31)* FEE CATEGORY 3.K AMOUNT ENCLOSED $1300.00

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
David B. Reader, Exec. Vice President		02/07/97

FOR NRC USE ONLY

TYPE OF FEE	FEE LOG	FEE CATEGORY	AMOUNT RECEIVED	CHECK NUMBER	COMMENTS
			$		124255 FEB 12 1997

APPROVED BY	DATE	
		FEB 11 1997 FAX REC'D

CIS-US, Inc.
Ten DeAngelo Drive
Bedford, MA 01730
(617) 275-7120
Subsidiary of CIS bio international

NRC FORM 313 ITEMS 7, 8, 9 AND 11 NOT APPLICABLE FOR THIS LICENSE.

5. RADIOACTIVE MATERIAL

a. Material	b. Chemical and/or physical form	c. Maximum amount
(1) Iodine-125	(1) ELSA-OSTEO *in vitro* RIA Kit	(1) 7.5 μCi [278 kBq] labeled activity at manufacture.

6. USE OF RADIOACTIVE MATERIAL

Item 5 a.-c.(1) Distribution by CIS-US pursuant to 10 CFR 32.71; to persons generally licensed under § 31.11 or equivalent general licensure of an Agreement State.

This license will not authorize possession or use of licensed material.[See 20-20973-01]

10. RADIATION SAFETY PROGRAM

Item 5 a.-c.(1)

1. The ELSA-OSTEO *in vitro* RIA Kit will be manufactured in France by CIS bio international (CBI), and supplied prepacked to CIS-US with package, labeling and instruction booklet as exhibited in **Attachment A**, page 3.

2. Transfer of kits to generally licensed persons will be subject to verification of recipient's registration or license pursuant to § 31.11(b) or equivalent general licensure of an Agreement State, in compliance with the requirements and methods specified in § 30.41(c) and (d).

1. NAME AND INTENDED USE

ELSA-OSTEO is a kit for the immunoradiometric assay of osteocalcin (Bone GLA Protein) in human serum and plasma.

2. INTRODUCTION

Osteocalcin or Bone GLA Protein (BGP) is a low molecular weight protein (5800 D). Synthesized by osteoblasts, it is specific to bone tissue and represents approximately 20 % of non-collagenous proteins. This 49 amino acids peptide contains 3 gamma-carboxy-glutamic acide (GLA) and has 2 potential sites for tryptic hydrolysis at the 19-20 and 43-44 residue levels. The resulting 1-19, 20-43, 44-49, 1-43 et 20-49 peptides may be the products of liver, kidney and plasmatic breakdown of the molecule. The physiological role of these fragments is unknown.

Physiologically, the osteocalcin level is elevated in children, particularly during the first year of life and during puberty when the evolution of the level is related to the rapidity of physical growth. For adults, the circulating osteocalcin level must be interpreted according to the age and sex of the patient.

In adults, there is a correlation between osteocalcin and the rate of bone turn over. Its presence is more marked in cases of hyper-remodeling (renal osteodystrophy, early hyperparathyroidism, hyperthyroidism, Paget's disease) and reduced in cases of hypo-remodeling (hypoparathyroidism, hypercalcemia resulting from bone metastasis and long term cortisone therapy).
When the processes of bone resorption and formation are out of phase (cortisone therapy, osteoporosis) serum osteocalcin provides a specific indicator of bone formation - i. e. of osteoblastic activity within the skeleton as a whole - and does not reflect bone resorption.

In primary osteoporosis, such as in the post-menopausal form when the phosphocalcic balance is normal, the assay of osteocalcin levels allows both classification of the disease according to regeneration rate (osteoporosis with high remodeling, osteoporosis with low remodeling) and a systematic follow-up of the patients treated.

During prolonged cortisone therapy, the osteocalcin measurement allows an evaluation of patient's osteoblastic activity. Here, a decline in osteoblastic activity is accompanied by a decrease of the osteocalcin level, with the decrease of osteocalcin level being in proportion to the amount of cortisone prescribed.

3. PRINCIPLE

ELSA-OSTEO is a solid-phase "sandwich" immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites. The first being coated on the ELSA solid phase ; the second, radiolabeled with iodine 125, is used as a tracer.

Osteocalcin molecules present in the standards or the samples to be tested are "sandwiched" between the two antibodies. Excess unbound tracer is easily removed during the procedure's washing step, and the ELSA retains only the adsorbed antibody/antigen/tracer antibody combination.

The amount of radioactivity bound to the ELSA is proportional to the amount of osteocalcin present at the beginning of the assay.

4. REAGENTS

Each kit contains enough reagents for 48 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
ELSA : ready for use. Monoclonal anti-human osteocalcin antibody coated on ELSA fixed to the bottom of the tube.	traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
ANTI OSTEO ^{125}I : ready for use. ^{125}I monoclonal anti-human osteocalcin antibody, buffer, animal proteins, sodium azide, red dye, non-immunized mice immunoglobulins. ≤ 278 kBq (≤ 7.5 µCi)	1 x 15 ml Tracer	2-8°C until the expiry date.
STANDARD 0 : lyophilized. Buffer, animal proteins. Reconstitute with 4 ml of distilled water.	1 qs 4 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**

STANDARDS : lyophilized. Buffer, animal proteins, human osteocalcin. 5 - 40 - 120 - 300 ng/ml*. Reconstitute with 0.5 ml of distilled water.	4 qs 0.5 ml vials	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C .**
CONTROL : lyophilized. Buffer, animal proteins, human osteocalcin. 20 ng/ml*. Reconstitute with 0.5 ml of distilled water.	1 qs 0.5 ml vial	2-8°C until the expiry date. After reconstitution : 1 month at - 20°C.**
TWEEN 20 : concentrated solution. Dilute 9 ml of Tween 20 in 3 liters of distilled water. Shake gently.	1 10 ml vial	2-8°C until the expiry date. After dilution, store in a capped container for 15 days maximum.
PLASTIC BAG	1	

(*) The values shown above are only target values : the true value of each standard or control is shown on its label.

(**) Standards and control should be frozen and thawed once.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Human tissue derivatives contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all human tissue derived products, including the samples to be assayed, must be treated as potentially infectious.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3 Handling precautions

Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbic contamination of the reagents or of the water used for washing.
Fully respect the incubation conditions and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma, haemolyzed or hyperlipemic samples should not be used. Heparin or EDTA plasma samples should be used, citrate as anticoagulant have to be discarded. If the test is to be carried out within 4 hours, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed.

Dilution

Should elevated osteocalcin levels be suspected, the O Standard found in the kit is used for dilution.
It is recommended that disposable plastic tubes be used when carrying out dilutions.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 50 µl, 300 µl, 500 µl and 4 ml (± 1%). Their calibration should be checked regularly.
Distilled water.
Disposable plastic tubes.
Vortex-type mixer.
Circular horizontal shaker.
Gamma scintillation counter calibrated for 125 iodine measurement.

Equipment suitable for this assay is available from CIS bio international ; information on request.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Standards and control must be reconstituted 15 minutes before use. Dispensing of the reagent into the ELSA tubes is also carried out at room temperature (18-25°C).

The assay requires the following groups of tubes :
O Standard group, for the determination of non-specific binding.
Standard groups, to establish the standard curve.
Control group for the control.
Sx groups, for the samples to be assayed.
It is recommended to performe the assay in triplicate for the standards and in duplicate for the samples.

Observe the order in which reagents are to be added :

Dispense 50 µl of standards, or control or samples to be assayed, into the corresponding-labeled ELSA tubes.
Add 300 µl of ^{125}I anti-human osteocalcin to each ELSA tube.
Gently mix each tube with a Vortex-type mixer.
Incubate for 2 h (± 5 mn) at room temperature (18-25°C) under agitation.

Wash the ELSA tubes as follows :

Aspirate the content of the tubes as completely as possible.
Add 3.0 ml of washing solution to each tube, and re-empty.
Repeat the process twice.
To obtain reliable and reproducible results, the different washing steps have to be correctly performed.
As much as possible of the incubation and washing solutions must be removed. If manual aspiration is used, the tip of the aspirating device must be placed right at the bottom of the tube.
Measure the radioactivity bound to the ELSA with a gamma scintillation counter.

8. QUALITY CONTROL

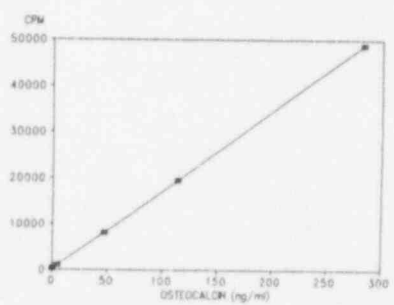
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.
Draw up the standard curve by plotting the standard's cpm against their concentrations.
Read the sample values directly from the curve, correcting the read value for the dilution factor, if necessary.

Typical standard curve (example only) : these data must under no circumstances be substituted for results obtained in the laboratory.

Tube groups	Mean cpm	Concentration ng/ml
Standard 0	205	0
Standard 1	1 064	5
Standard 2	8 094	47
Standard 3	19 532	114
Standard 4	48 735	283
Control	3 510	20
Sample 1	12 105	72
Sample 2	29 090	171



10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results. Do not attempt to extrapolate sample values beyond the last standard. Dilute the samples concerned and retest.

11. EXPECTED VALUES

Each laboratory should establish its own range of normal values. Values shown below are only an example. The chart below shows the distribution of values obtained in presumably normal adult subjects.

	Age (years)	Number of cases	Mean ng/ml	Median ng/ml	Extreme values ng/ml
Males	20 - 30	48	23.8	22.0	11.3 - 37.0
	31 - 40	51	21.5	19.4	10.7 - 34.1
	41 - 50	49	20.3	19.9	5.2 - 34.5
	51 - 60	91	18.7	18.6	6.3 - 30.7
	61 - 70	60	19.1	19.2	8.8 - 29.7
Females	20 - 30	70	21.8	21.1	8.8 - 39.4
	31 - 40	87	17.1	16.2	7.7 - 31.9
	41 - 50	74	15.7	15.0	8.0 - 36.0
	51 - 60	85	24.4	22.6	8.0 - 50.5
	61 - 70	32	24.4	24.3	12.9 - 55.9

The values have to take into account age and sex.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 2 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 20 different series.

Sample	Mean ng/ml	Within-run CV %	Between-run CV %
1	21.9	3.8	5.2
2	183.9	3.9	4.5

12.2. Recovery test

Known quantities of human osteocalcin were added to human sera. The recovery percentages of human osteocalcin in the samples ranged from 95 to 105 %.

12.3. Dilution test

Ten samples with high levels were diluted with the recovery percentages ranging from 95 to 105 %.

12.4. Specificity

ELSA-OSTEO measures the 1-49 human osteocalcin (carboxylated or decarboxylated) and human osteocalcin peptide 1-43.
On the other hand, human osteocalcin peptides 7-19, 25-37 and 37-49 do not interfere in the ELSA-OSTEO assay for concentration < 10 000 ng/ml.

12.5. Detection Limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95 %. It has been assessed as being 0.4 ng/ml.

ASSAY FLOW-CHART

Tubes	Standards Control Samples µl	^{125}I anti-human osteocalcin µl	Mix Incubate 2 H ± 5 mn à 18-25°C under agitation Wash 3 times with the washing solution	Count
Standards	50	300		
Control or Samples	50	300		

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Octobre 1994 - Modèle 05

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For Research Use Only - Not for use in diagnostic procedures

The radioactive material in this *in vitro* RIA kit may be received, acquired, possessed and used only by physicians, veterinarians i the practice of veterinary medicine, clinical laboratories or hospitals and only for *in vitro* clinical or laboratory tests not involvi internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisitio use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State wi which the Commission has entered into an agreement for the exercise of regulatory authority.

Distributed by CIS-US, Inc., 10 DeAngelo Dr., Bedford, MA 01730

ELSA-OSTEO

CIS bio international

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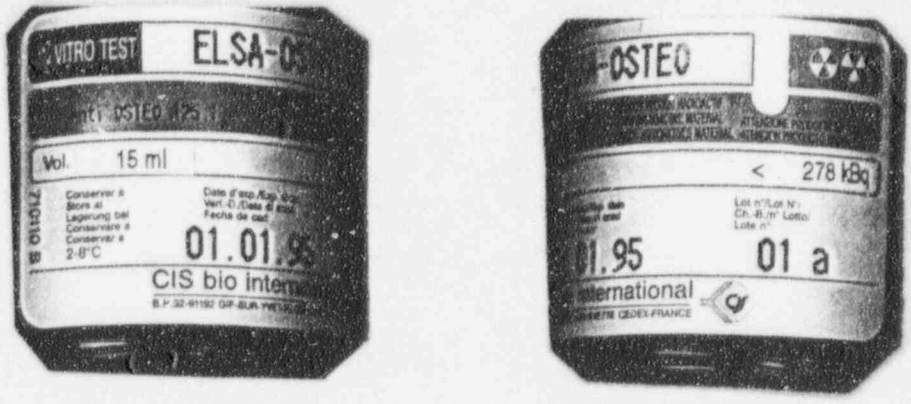
ATTACHMENT A

Exhibit: ELSA-OSTEO RIA KIT

Side 1

Side 2 Kit Cover Label (over)

ANTI OSTEO ^{125}I TRACER VIAL LABEL



INSTRUCTION BOOKLET

BETWEEN:

LICENSE FEE MANAGEMENT BRANCH, ARM
AND
REGIONAL LICENSING SECTIONS

(FOR LFMS USE)
INFORMATION FROM LTS

PROGRAM CODE: 03244
STATUS CODE: 3
FEE CATEGORY: _____
EXP. DATE: 0
FEE COMMENTS: _____
DECOM FIN ASSUR REQD: _

LICENSE FEE TRANSMITTAL

A. REGION I

1. APPLICATION ATTACHED
APPLICANT/LICENSEE: CIS-US, INC.
RECEIVED DATE: 970211
DOCKET NO: 3034394
CONTROL NO.: 124255
LICENSE NO.:
ACTION TYPE: NEW LICENSE

2. FEE ATTACHED
AMOUNT: $1300.00
CHECK NO.: 019670

3. COMMENTS

SIGNED M. A. Perkins
DATE 2/12/97

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

1. FEE CATEGORY AND AMOUNT: 3K $1,300

2. CORRECT FEE PAID. APPLICATION MAY BE PROCESSED FOR:
AMENDMENT _____
RENEWAL _____
LICENSE /

3. OTHER _____
_____

SIGNED _____
DATE _____

I
Log Feb 9 (97)
Number
Check No. 019670
Amount $1,300
Fee Category 3K
Type of Fee APP
Date Check Rec'd 2/21/97
Date Completed
By: BO

07 for 2/21/97

1997 FEB 20 AM 9:41