Rat Osteocalcin IRMA Kit

Immutopics

Immunoradiometric Assay (IRMA) for the Quantitative Determination of Rat Osteocalcin Levels in Serum, Plasma or Cell Culture Media

Immutopics, Inc.

For RESEARCH Use Only
Not for use in diagnostic procedures

100 Test Kit

Cat.# 50-1500

CAUTION: Radioactive Materials
Not for Internal or External Use in Humans or Animals

Store at 2 - 8ºC Upon Receipt

INTENDED USE

This kit is intended for research use only in the determination of rat osteocalcin levels in serum, plasma, or cell culture media.

INTRODUCTION

Rat osteocalcin, a 50 amino acid peptide, is the major noncollagen protein found in rat bone. It contains three gamma-carboxyglutamic acid (GLA) residues at positions 17, 21, and 24 and is, therefore, also known as bone gla-protein or BGP. The exact biological function of osteocalcin is not known but the three gamma-carboxyglutamic acid residues confer on it a very strong ability to bind to hydroxyapatite and calcium.

Vitamin K is essential for the biosynthesis of osteocalcin which is stimulated by 1,25-dihydroxyvitamin D. Osteocalcin is synthesized by osteoblasts during the process of bone formation and mostly incorporated into bone matrix with some escaping into the blood. Since the half-life in blood is relatively short (about 5 minutes) the osteocalcin level in blood reflects new protein synthesis and therefore its measurement provides a valuable tool for assessing skeletal metabolism. As a product unique to the osteoblast, it also represents the activity of the cell responsible for the formation of bone.

TEST PRINCIPLE

The Rat Osteocalcin IRMA Kit is a two - site immunoradiometric assay (IRMA) for the measurement of rat osteocalcin in serum, plasma or cell culture media. Two different antibodies to rat osteocalcin are used in the assay. An affinity purified polyclonal goat antibody recognizing the mid-region C-terminal portion of the molecule is immobilized onto plastic beads for capture and another affinity purified polyclonal goat antibody recognizing the amino terminal portion of the molecule is radiolabeled for detection.

A sample containing rat osteocalcin is incubated simultaneously with an antibody coated bead and the ^{125}I labeled antibody. Osteocalcin contained in the sample is immunologically bound by the immobilized antibody and the radiolabeled antibody to form a "sandwich" complex:

Bead/Anti-Rat Osteocalcin — Rat Osteocalcin — ^{125}I Anti-Rat Osteocalcin

At the end of the incubation period, the bead is washed to remove any unbound labeled antibody and other components. The radioactivity bound to the bead is then measured in a gamma counter. The radioactivity of the antibody complex bound to the bead is directly proportional to the amount of rat osteocalcin in the sample. A standard curve is generated by plotting the CPM versus the respective rat osteocalcin concentration for each standard on logarithmic scales. The concentration of rat osteocalcin in the samples is determined directly from this curve.

REAGENTS: Preparation and Storage

Store the kit at 2-8ºC upon receipt. **Store the standards and controls at -20ºC or below after reconstitution.** For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature and mix by gentle swirling and inversion. Reagents from different kit lot numbers should not be combined or interchanged.

1. **RAT OSTEOCALCIN ANTIBODY COATED BEADS (40-1510)**
   One container of 100 polystyrene beads (6.4 mm diameter) coated with antibody to rat osteocalcin plus desiccant.

2. **^{125}I LABELED RAT OSTEOCALCIN ANTIBODY (40-1520)**
   Two vials each containing 10.5 mL of ^{125}I labeled anti-rat osteocalcin in 0.25M phosphate buffered saline and 0.025 M EDTA with protein stabilizers and 0.1% sodium azide. Each vial contains less than 10 uCi (370 kBq) of radioactivity.

3. **RAT OSTEOCALCIN STANDARDS (40-1531 to 40-1536)**
   Six vials, five of which contain synthetic rat osteocalcin (1-50) lyophilized in a protein matrix with 0.1% sodium azide. **Refer to vial label for exact concentration.** The zero standard is also used as the sample diluent and is supplied as a 20 mL ready-to-use liquid. Before use reconstitute each of the other five vials of standards with 1.0 mL of deionized water. Allow the vials to sit for approximately 15 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

   Use the standards immediately after reconstitution; freeze the unused portion for later use. The zero standard may be stored at 2 - 8°C. After reconstitution the standards are stable until the expiration date on the kit box when stored at -20ºC or below with up to 3 freeze/thaw cycles.

4. **RAT OSTEOCALCIN CONTROLS I & II (40-1541 & 40-1542)**
   Two vials each containing rat osteocalcin (1-50) lyophilized in a protein matrix with 0.1% sodium azide. **Refer to vial label for control ranges.** Before use reconstitute each control with 1.0 mL of deionized water. Allow the vials to sit for approximately 15 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

   Use the controls immediately after reconstitution; freeze the unused portion for later use. After reconstitution the controls are stable until the expiration date on the kit box when stored at -20ºC or below with up to 3 freeze/thaw cycles.

5. **WASH CONCENTRATE (40-0050)**
   One bottle containing 30 mL of a 30 fold concentrate. Before use dilute the contents to 900 mL with deionized water and mix well. Upon dilution this yields a working wash solution containing a surfactant in 0.01M phosphate buffered saline with 0.05 % sodium azide. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

Some of the reagents in this kit contain sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup (Manual Guide-Safety Management No. CDC-22, Center for Disease Control, Atlanta, Georgia, April 30, 1976).

For practitioners or institutions receiving radioisotopes under a general license:

This radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical or research laboratories or hospitals, and is only for in vitro 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 the general license of the U.S. Nuclear Regulatory Commission or of the state with which the Commission has entered into an agreement for the exercise of regulatory authority.

1. Storage of radioactive material should be limited to a specifically designated area.
2. Access to radioactive materials must be limited to authorized personnel only.
3. Do not pipette radioactive material by mouth.
4. Do not eat or drink within designated radioactive work area.
5. Areas where spills may occur should be wiped up and then washed with an alkali detergent or radiological decontamination solution. Any glassware used must be rinsed completely with water before washing with other laboratory glassware.

For practitioners or institutions receiving radioisotopes under a specific license:

The receipt, use, transfer and disposal are subject to the regulations and conditions of your specific license.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Polystyrene or polypropylene test tubes, 12 x 75 mm.
2. Test tube rack.
3. Marking pen for labeling tubes.
4. 1.0 mL volumetric pipette for reconstituting standards and controls.
5. Precision pipets capable of delivering 20 µL, 100 µL, 200 µL, and 400 µL.
6. Forceps or suitable bead dispenser.
7. Parafilm® or equivalent for covering tubes.
8. Repeating dispenser suitable for delivering 2.0 mL.
9. Aspiration device or suitable bead washer.
10. Container for storage of wash solution.
11. Gamma counter.
12. Deionized water.
13. Vortex mixer.
14. Timer.

SPECIMEN COLLECTION

Measurement of the rat osteocalcin concentration may be made on serum, plasma, or cell culture media. Since serum and plasma samples must be diluted 1:21 prior to assay only twenty microliters are required to assay the sample in duplicate. If obtaining serum, collect blood and allow it to clot at room temperature. Centrifuge the sample and separate the serum, plasma, or media from the cells. Samples should be assayed immediately or stored frozen at -20ºC or below. Avoid repeated freezing and thawing of specimens.

ASSAY PROCEDURE

1. **Dilute both controls and each serum or plasma sample 1:21 prior to assay.** (Standards do not require dilution and are ready-to-use after reconstitution.) For sample dilution pipette 20 µL of sample and 400 µL of zero standard into appropriately labeled tubes and vortex. Cell culture media samples may have to be diluted differently to obtain optimum results.
2. Pipet 100 µL of standard, diluted control or diluted sample into appropriately labeled tubes.
3. Pipet 200 µL of ^{125}I Labeled Rat Osteocalcin Antibody into all tubes.
4. Vortex all tubes.
5. Using forceps or appropriate bead dispenser, add one bead to each tube. Tilt tube rack to approximately a 30 degree angle to prevent splashing. Cover tube rack with Parafilm® or equivalent.
6. Incubate tubes at room temperature for 18 to 24 hours.
7. Aspirate the contents of each tube. Wash beads three times by dispensing 2 mL of wash solution into each tube and then completely aspirating the contents.
8. Count each tube in a gamma counter for one minute and record the counts.

PROCEDURAL NOTES

1. It is recommended that standards, controls and samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.
2. The sample and the ^{125}I Labeled Rat Osteocalcin Antibody should be pipetted carefully into the bottom one-fourth of the tube.
3. The washing step is an important part of the total assay procedure. Accurate dispensing of the wash solution and thorough and complete aspiration of the tube contents is essential. The length of time the wash solution sits in each tube is also an important factor. The washing procedure should be performed such that this timing is as consistent as possible.
4. If data reduction requires total count tubes, label duplicate tubes appropriately and pipet 200 µL of the ^{125}I Labeled Rat Osteocalcin Antibody into each tube and cap.

5. Samples with values greater than the highest standard should be further diluted with the 0 ng/mL Standard and reassayed. Multiply the result by the dilution factor. (See Limitations, # 1 and # 2).
6. Plasma or cell culture media samples may contain fibrin clots or cellular debris. Freeze/thaw of plasma samples may accelerate clot formation. These samples must be centrifuged and decanted prior to assay to remove all particulate material which can cause random high non-specific binding on tube or bead surface.

CALCULATION OF RESULTS

The standard curve is generated using the rat osteocalcin standards contained in the kit. **Refer to individual vial labels for exact concentrations.** Generate the curve as follows:

1. Calculate the average CPM for each pair of duplicate assay tubes.
2. Subtract the average CPM of the 0 ng/mL Standard from all other average CPMs to obtain corrected CPM.
3. The standard curve is generated by plotting the corrected CPM of each standard level on the ordinate against the standard concentration on the abscissa using log-log paper. Appropriate computer assisted data reduction programs may also be used for calculation of rat osteocalcin results.

The rat osteocalcin concentration of the diluted controls and diluted samples are read directly from the standard curve using their respective corrected CPM. Samples having a corrected CPM between the 0 ng/mL Standard and the next highest standard should be calculated by the formula:

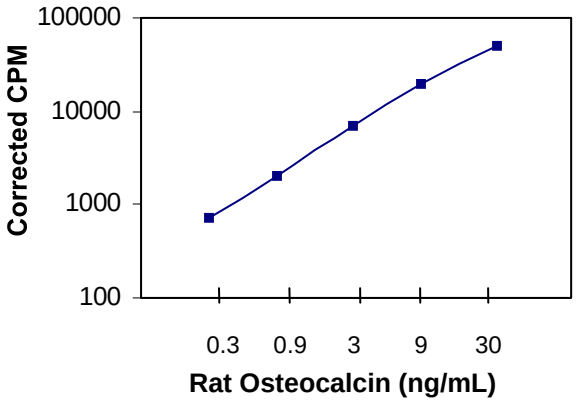
$$\text{Value of unknown} = \frac{\text{Corrected CPM (unknown)}}{\text{Corrected CPM (2nd Std)}} \times \text{Value of the 2nd Std.}$$

To obtain final rat osteocalcin concentrations for controls and samples multiply the observed values by the dilution factor.

EXAMPLE DATA AND STANDARD CURVE

The following are representative examples of data and the resulting standard curve. **This curve should not be used in lieu of a standard curve run with each assay.**

Tube #	Tube I.D.	CPM	Average CPM	Corrected CPM	Result ng/mL	Corrected Result ng/mL
1	0 ng/mL	394				
2		362	378			
3	0.3 ng/mL	2613				
4		2724	2669	2291		
5	0.9 ng/mL	5411				
6		5648	5530	5152		
7	3 ng/mL	12139				
8		12999	12569	12191		
9	9 ng/mL	27986				
10		28794	28390	28012		
11	30 ng/mL	45366				
12		47668	46517	46139		
13	Control I	7255				
14		7666	7461	7083	1.43	30
15	Control II	15702				
16		16675	16189	15811	4.15	87
17	Sample 1	5694				
18		5870	5782	5405	0.96	20
19	Sample 2	12407				
20		12314	12361	11983	2.93	62



LIMITATIONS OF THE PROCEDURE

1. The lowest concentration of rat osteocalcin measurable is 0.01 ng/mL (assay sensitivity) and the highest concentration of rat osteocalcin measurable is the value of the highest standard. Due to the inherent statistical variation at the highest standard, as observed in any IRMA, a sample with a concentration exceeding the highest standard may, on occasion, read less than the highest standard.
2. The reagents in this Rat Osteocalcin IRMA Kit have been optimized so that the high dose “hook effect” is not a problem for diluted samples with elevated rat osteocalcin values. Diluted samples with rat osteocalcin levels between the highest standard and 1,000 ng/mL will read greater than the highest standard and should be further diluted 1:10 with the 0 ng/mL Standard and reassayed for correct values.
3. Grossly lipemic serum or plasma samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
4. Differences in protein concentration and protein type between samples and standards in an immunoassay contribute to "protein effects" and dose biases. When measuring low protein concentration culture media samples against high protein concentration standards, it is recommended that like samples be assayed together in the same assay to minimize this bias.

QUALITY CONTROL

To assure the validity of the results, each assay should include adequate controls with known levels of rat osteocalcin. Immutopics recommends that all assays include the laboratory’s own rat osteocalcin controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS:

SENSITIVITY

The sensitivity of the Rat Osteocalcin IRMA Assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 ng/mL Standard is 0.01ng/mL.

PRECISION

To assess intra-assay precision the mean and coefficient of variation were calculated from 20 duplicate determinations of two diluted samples each performed in a single assay.

Observed Mean Value (ng/mL)	Coefficient of Variation
1.5	2.0 %
4.3	2.3 %

To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two diluted samples performed in 20 assays.

Observed Mean Value (ng/mL)	Coefficient of Variation
1.4	5.0 %
4.0	4.0 %

PARALLELISM

Rat plasma samples were diluted with the 0 ng/mL Standard and assayed. Results, corrected for dilution, in ng/mL are as follows:

SAMPLE	DILUTION	CORR. OBSERVED VALUE	CORR. EXPECTED VALUE	% O/E
Plasma	1:10	40.9	39.1	105
	1:20	39.1	39.1	100
	1:40	39.0	39.1	100
	1:80	38.4	39.1	98
Plasma	1:10	118	115	103
	1:20	115	115	100
	1:40	106	115	92
	1:80	113	115	98
Plasma	1:10	32.0	29.2	110
	1:20	29.2	29.2	100
	1:40	31.2	29.2	107
	1:80	28.8	29.2	99

RECOVERY

Various amounts of rat osteocalcin were added to different rat serum and/or plasma samples and assayed. Results, corrected for dilution, in ng/mL are as follows:

SAMPLE	ORIG. VALUE	AMOUNT ADDED	OBSERVED VALUE	EXPECTED VALUE	% O/E
1	28.1	14.0	43.2	42.1	103
		28.1	57.9	56.2	103
		42.1	76.5	70.2	109
2	18.0	16.6	35.0	34.6	101
		33.1	53.5	51.1	105
		49.7	67.3	67.7	99

CROSS-REACTIVITY

Both human osteocalcin and mouse osteocalcin were diluted in an osteocalcin-free protein matrix and measured in the Rat Osteocalcin IRMA Kit. The results show zero cross-reactivity from either of these species.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Immutopics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Immutopics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights which vary from state to state.

REFERENCES

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4. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, and Karsenty G, "Increased bone formation in osteocalcin-deficient mice", *Nature*, Vol 382, 1 Aug 1996, 448-452.

CLIENT SERVICES

To place an order or for technical assistance, contact Immutopics International at (800) 681-6665 or (949) 369-9207 or FAX to (949) 369-9405 or e-mail: info@immutopicsintl.com.

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