# Rat Calcitonin IRMA Kit

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

Immutopics

Immutopics, Inc.

**For RESEARCH Use Only**
**Not for use in diagnostic procedures**

**100 Test Kit**

**Cat.# 50-5000**

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

**Store at 2 - 8ºC Upon Receipt**

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## INTENDED USE

This kit is intended for research use only in the determination of rat calcitonin levels in serum, plasma, or cell culture media. **This assay is also useful in the determination of mouse calcitonin levels.**

## INTRODUCTION

Calcitonin is a 32 amino acid peptide hormone with a 1-7 disulfide bridge synthesized primarily by thyroidal C-cells. Other tissue sources of calcitonin have been identified but they are not likely to contribute to the circulating concentration. Calcitonin circulates in various multiple molecular forms, the physiological significance of which is unknown. These consist of as many as four or five potentially immunoreactive forms which are probably polymers of calcitonin or precursor forms.

Calcitonin secretion is regulated by changes in plasma calcium concentration. Acute increases in calcium concentration create an increase in peptide secretion and acute decreases produce a corresponding decrease in secretion.

The primary biological action of calcitonin is the inhibition of osteoclastic bone resorption. The entire molecule with an intact 1-7 disulfide bridge is required for full biologic activity. Both nonmonomeric and monomeric forms of circulating calcitonin have biologic activity.

The metabolism of calcitonin is a complex process involving many organ systems. The kidney, however, appears to be the important organ of clearance with hormone inactivation more important than renal excretion.

## TEST PRINCIPLE

The Rat Calcitonin IRMA Kit is a two - site immunoradiometric assay (IRMA) for the measurement of rat calcitonin in serum, plasma or cell culture media. Two different antibodies to rat calcitonin are used in the assay. A monoclonal antibody is immobilized onto plastic beads to capture the calcitonin molecules and an affinity purified polyclonal goat antibody is radiolabeled for detection.

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

Bead/Anti-Rat Calcitonin — Rat Calcitonin — $^{125}$I Anti-Rat Calcitonin

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 calcitonin in the sample. A standard curve is generated by plotting the CPM versus the respective rat calcitonin concentration for each standard on logarithmic scales. The concentration of rat calcitonin 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 CALCITONIN ANTIBODY COATED BEADS (40-5010)**
   One container of 100 polystyrene beads (6.4 mm diameter) coated with monoclonal antibody to rat calcitonin plus desiccant.

2. **$^{125}$I LABELED RAT CALCITONIN ANTIBODY (40-5020)**
   Two vials each containing 5.5 mL of $^{125}$I labeled anti-rat calcitonin in 0.6M phosphate buffered saline with protein stabilizers and 0.1% sodium azide. Each vial contains less than 10 uCi (370 kBq) of radioactivity.

3. **RAT CALCITONIN STANDARDS (40-5031 to 40-5036)**
   Six vials each containing synthetic rat calcitonin (1-32) lyophilized in a protein matrix with 0.1% sodium azide. **Refer to vial label for exact concentration.** Before use reconstitute the vial with the rat calcitonin concentration of 0 pg/mL with 4.0 mL of deionized water. Before use reconstitute each of the other five vials of standards with 2.0 mL of deionized water. Allow the vials to sit for approximately 20 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. 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 CALCITONIN CONTROLS I & II (40-5041 & 40-5042)**
   Two vials each containing rat calcitonin (1-32) lyophilized in a protein matrix with 0.1% sodium azide. **Refer to vial label for control ranges.** Before use reconstitute each control with 2.0 mL of deionized water. Allow the vials to sit for approximately 20 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.

6. **RAT CALCITONIN SAMPLE DILUENT (Optional reagent, must be ordered separately using catalog # 30-5031)**
   One bottle containing 20 mL of a protein matrix with 0.1% sodium azide in liquid, ready-to-use form. This reagent should be stored at 2 - 8ºC and is stable until the expiration date on the bottle.

## 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 tubes, 12 x 75 mm.
2. Test tube rack.
3. Marking pen for labeling tubes.
4. 2.0 mL and 4.0 mL volumetric pipettes for reconstituting standards and controls.
5. Precision pipets capable of delivering 100 µL and 200 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 calcitonin concentration may be made using serum, plasma, or cell culture media. Four hundred microliters of serum, plasma, or culture media 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. Pipet 200 µL of standard, control or sample into appropriately labeled tubes.
2. Pipet 100 µL of $^{125}$I Labeled Rat Calcitonin Antibody into all tubes.
3. Vortex all tubes.
4. 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.
5. Incubate tubes at room temperature for 18 to 24 hours.
6. 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.
7. Count each tube in a gamma counter for one minute and record the counts.

## PROCEDURAL NOTES

1. It is recommended that all 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 Calcitonin 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 100 µL of the $^{125}$I Labeled Rat Calcitonin Antibody into each tube and cap.
5. Samples with values greater than the highest standard should be diluted 1:10 with the 0 pg/mL Standard or the optional Sample Diluent reagent and reassayed. Multiply the result by 10. (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 calcitonin 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 pg/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 calcitonin results.

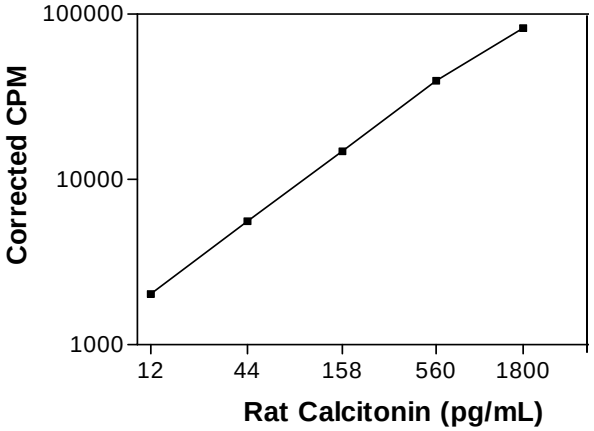
The rat calcitonin concentration of the controls and samples are read directly from the standard curve using their respective corrected CPM. Samples having a corrected CPM between the 0 pg/mL Standard and the next highest standard should be calculated by the formula:

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

## 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 | Results pg/mL |
|---|---|---|---|---|---|
| 1 | 0 pg/mL | 484 | | | |
| 2 | | 469 | 477 | | |
| 3 | 12 pg/mL | 2536 | | | |
| 4 | | 2478 | 2507 | 2030 | |
| 5 | 44 pg/mL | 6059 | | | |
| 6 | | 6076 | 6068 | 5591 | |
| 7 | 158 pg/mL | 15443 | | | |
| 8 | | 15185 | 15314 | 14837 | |
| 9 | 560 pg/mL | 40015 | | | |
| 10 | | 39963 | 39989 | 39512 | |
| 11 | 1800 pg/mL | 84300 | | | |
| 12 | | 81422 | 82861 | 82384 | |
| 13 | Control I | 4314 | | | |
| 14 | | 4168 | 4241 | 3764 | 27 |
| 15 | Control II | 28130 | | | |
| 16 | | 27042 | 27586 | 27109 | 338 |
| 17 | Sample 1 | 5698 | | | |
| 18 | | 5877 | 5788 | 5311 | 41 |
| 19 | Sample 2 | 16559 | | | |
| 20 | | 16734 | 16647 | 16170 | 176 |




## LIMITATIONS OF THE PROCEDURE

1. The lowest concentration of rat calcitonin measurable is 0.6 pg/mL (assay sensitivity) and the highest concentration of rat calcitonin measurable without dilution 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 Calcitonin IRMA Kit have been optimized so that the high dose “hook effect” is not a problem for samples with elevated rat calcitonin values. Samples with rat calcitonin levels between the highest standard and 300,000 pg/mL will read greater than the highest standard and should be diluted 1:10 with the 0 pg/mL Standard or the optional Sample Diluent reagent 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 calcitonin. Immutopics recommends that all assays include the laboratory’s own rat calcitonin controls in addition to those provided with this kit.

## OBSERVED VALUES

Several rat plasma samples collected under varying physiological conditions were assayed by Immutopics to demonstrate relative assay specificity. Due to variable research conditions a normal range has not been defined. Each laboratory should establish it's own normal range.

Normal rats (n=16) with ionized plasma calcium values between 1.23 and 1.43 mM had calcitonin levels of 1.2 to 19.7 pg/mL. Thyroparathyroidectomized rats (n=12) had undetectable calcitonin levels when sampled 4 hours or later after surgery. Elevated calcitonin levels were induced by acute injection of calcium in rats (n=4). After 10 minutes ionized plasma calcium values were above 1.67 mM and calcitonin levels measured between 19 and 176 pg/mL.

Culture media from *in vitro* incubations of neonatal rat thyroparathyroid complexes demonstrated a similar response to acute calcium stimulation.

## PERFORMANCE CHARACTERISTICS:

### SENSITIVITY
The sensitivity of the Rat Calcitonin IRMA assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 0.6 pg/mL.

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

| Mean Value (pg/mL) | Coefficient of Variation |
|---|---|
| 33 | 3.0% |
| 406 | 1.8% |

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

| Mean Value (pg/mL) | Coefficient of Variation |
|---|---|
| 33 | 3.5% |
| 416 | 2.8% |

### PARALLELISM
Rat plasma and rat thyroparathyroid cell culture media samples were diluted with the 0 pg/mL Standard and assayed. Results in pg/mL are as follows:

| SAMPLE | DILUTION | OBSERVED VALUE | EXPECTED VALUE | % O/E |
|---|---|---|---|---|
| Plasma | undiluted | 176 | | |
| | 1:2 | 92 | 88 | 105 |
| | 1:4 | 49 | 44 | 111 |
| | 1:8 | 23 | 22 | 105 |
| Plasma | undiluted | 102 | | |
| | 1:2 | 51 | 51 | 100 |
| | 1:4 | 25 | 26 | 96 |
| | 1:8 | 12 | 13 | 92 |
| Media | undiluted | 749 | | |
| | 1:2 | 418 | 375 | 111 |
| | 1:4 | 209 | 187 | 112 |
| | 1:8 | 98 | 94 | 104 |

### RECOVERY
Various amounts of rat calcitonin were added to different rat plasma samples and assayed. Results in pg/mL are as follows:

| SAMPLE | ORIG. VALUE | AMOUNT ADDED | OBSERVED VALUE | EXPECTED VALUE | % O/E |
|---|---|---|---|---|---|
| 1 | 30 | 78 | 87 | 108 | 81 |
| | | 156 | 165 | 186 | 89 |
| | | 235 | 248 | 265 | 94 |
| 2 | 110 | 19 | 128 | 129 | 99 |
| | | 38 | 138 | 148 | 93 |
| | | 57 | 157 | 167 | 94 |

### CROSS-REACTIVITY
The following cross-reactants were diluted in a calcitonin-free protein matrix up to levels of 10,000 pg/mL and measured using the Rat Calcitonin IRMA Kit. The results are expressed as % cross-reactivity relative to the rat calcitonin standards contained in the kit.

| CROSS-REACTANT MEASURED | CROSS-REACTIVITY: |
|---|---|
| human calcitonin | 12.5 % |
| chicken calcitonin | <0.001 % |
| eel calcitonin | <0.001 % |
| porcine calcitonin | <0.001 % |
| salmon calcitonin | <0.001 % |
| mouse calcitonin | results similar to rat; unable to quantify because purified peptide is not available |

### 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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### 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@immutopisintl.com.


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