Rat Insulin

250 Tubes

Cat. # RI-13K

RAT INSULIN RIA KIT
250 TUBES (Cat. # RI-13K)

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RAT INSULIN RIA KIT
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I. INTENDED USE

This Rat Insulin Radioimmunoassay (RIA) Kit is for the quantitative determination of Rat Insulin in serum, plasma, and other tissue culture media. The primary antibody was raised in guinea pigs against highly purified Rat Insulin. Sensitivity of 0.081 ng/mL + 2 SD can easily be achieved when using a 100 µL serum or plasma sample in an overnight, equilibrium assay (300 µL Total Volume). An alternative room temperature incubation for two hours may be used to shorten the assay to provide results within one day.

This kit is for Research Use Only. Not for Use in Diagnostic Procedures.

II. PRINCIPLES OF PROCEDURE

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity. The EMD Millipore Rat Insulin assay utilizes ^{125}I-labeled insulin and an Insulin antiserum to determine the level of Rat Insulin in serum, plasma or tissue culture media by the double antibody/PEG technique.

III. REAGENTS SUPPLIED

Each kit is sufficient to run 250 tubes and contains the following reagents.

A. Assay Buffer
0.05M Phosphosaline, pH 7.4, containing 0.025M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA
Quantity: 40 mL/vial
Preparation: Ready to use

B. Insulin Antibody
Insulin Serum in Assay Buffer
Quantity: 26 mL/vial
Preparation: Ready to use

C. ^{125}I-Insulin
^{125}I-Insulin Label, HPLC purified (specific activity 367 µCi/µg)
Lyophilized for stability. Freshly iodinated label contains <5 µCi (<185 kBq), calibrated to the 1st Monday of each month.
Quantity: 27 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with entire contents of Label Hydrating Buffer. Allow to sit at room temperature for 30 minutes, with occasional gentle mixing.

D. Label Hydrating Buffer
Assay Buffer containing Normal Guinea Pig IgG as carrier. Use the entire content to hydrate ^{125}I-Insulin.
Quantity: 27 mL/vial
Preparation: Ready to use

E. Rat Insulin Standards
Purified Rat Insulin in Insulin Standard Buffer at the following concentration: 10.0 ng/mL
Quantity: 2 mL/vial
Preparation: Ready to use

F. Quality Controls 1 & 2
Purified Rat Insulin in Assay Buffer
Quantity: 1 mL/vial
Preparation: Ready to use

G. Precipitating Reagent
Goat anti-Guinea Pig IgG Serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA, 0.08% Sodium Azide
Quantity: 260 mL/vial
Preparation: Ready to use, chill to 4°C

IV. STORAGE AND STABILITY

Refrigerate all reagents between 2 and 8°C for short term storage. For prolonged storage (>2 weeks), freeze at ≤ -20°C. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at ≤ -20°C. Do not mix reagents from different kits unless they have the same lot number.

V. REAGENT PRECAUTIONS

A. Radioactive Materials

This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in vitro research 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 of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer (RSO) is ultimately responsible for the safe handling and use of radioactive material.

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, clothing and immediate area surrounding the workstation for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.
5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

B. Sodium Azide

Sodium Azide has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Note: See Full Labels of Hazardous components on next page.

V. REAGENT PRECAUTIONS (continued)

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
^{125} I-Insulin Tracer	9011		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Insulin Antibody	1013-K		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Precipitating Reagent	PR-UV		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the investigator finds that the pellet formation is acceptably stable in their system.)
2. 100 µL pipette with disposable tips
3. 100 µL & 1.0 mL repeating dispenser
4. Refrigerated swing-bucket centrifuge capable of developing 2,000 - 3,000xg. (Use of fixed-angle buckets is not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter

VII. SPECIMEN COLLECTION AND STORAGE

1. A maximum of 100 µL per assay tube of serum or plasma can be used, although 50 µL per assay tube is adequate for most applications. Tissue culture and other media may also be used.
2. Care must be taken when using heparin as an anticoagulant, since an excess will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.
3. Specimens can be stored at 4°C if they will be tested within 24 hours of collection. For longer storage, specimens should be stored at ≤ -20°C. Avoid multiple (>5) freeze/thaw cycles.
4. Avoid using samples with gross hemolysis or lipemia.

VIII. ASSAY PROCEDURE

For optimal results, accurate pipetting and adherence to the protocol are recommended.

Standard Preparation

Use care in opening the Standard vial.

Label six glass tubes 1, 2, 3, 4, and 5. Add 1.0 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 1.0 mL of the 10 ng/mL standard to tube 1, mix well and transfer 1.0 mL of tube 1 to tube 2, mix well and transfer 1.0 mL of tube 2 to tube 3, mix well and transfer 1.0 mL of tube 3 to tube 4, mix well and transfer 1.0 mL of tube 4 to tube 5, mix well and transfer 1.0 mL of tube 5 to tube 6, mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Standard Concentration	Volume of Assay Buffer to Add	Volume of Standard to Add
1	1.0 mL of 5 ng/mL	1.0 mL	1.0 mL of 10 ng/mL
2	1.0 mL of 2.5 ng/mL	1.0 mL	1.0 mL of 5 ng/mL
3	1.0 mL of 1.25 ng/mL	1.0 mL	1.0 mL of 2.5 ng/mL
4	1.0 mL of 0.625 ng/mL	1.0 mL	1.0 mL of 1.25 ng/mL
5	1.0 mL of 0.313 ng/mL	1.0 mL	1.0 mL of 0.625 ng/mL
6	1.0 mL of 0.156 ng/mL	1.0 mL	1.0 mL of 0.313 ng/mL

A. Assay Set-Up, Day One

1. Pipette 200 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4) and 100 µL to Reference (Bo) tubes (5-6). No buffer is added to tubes 7 through the end of the assay.
2. Pipette 100 µL of Standards and Quality Controls in duplicate (see flow chart).
3. Pipette 100 µL of each sample in duplicate.

   (NOTE: Smaller volumes of sample may be used when Insulin concentrations are anticipated to be elevated or when sample size is limited. Additional Assay Buffer should be added to compensate for the difference so that the volume is equivalent to 100 µL, e.g., when using 50 µL of sample, add 50 µL of Assay Buffer). Refer to Section IX for calculation modification.
4. Pipette 100 µL of hydrated ^{125}I-Insulin to all tubes. Important: For preparation, see Section III, Part C.
5. Pipette 100 µL of Insulin antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

B. Day Two

7. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).
8. Vortex and incubate 20 minutes at 4°C.
9. Centrifuge, 4°C, all tubes except Total Count tubes (1-2) for 20 minutes at 2,000-3,000 xg.
   NOTE: If less than 2,000 xg is used or if slipped pellets have been observed in previous runs, the time of centrifugation must be increased to obtain a firm pellet (e.g., 40 minutes). Multiple centrifuge runs within an assay must be consistent.

   Conversion of rpm to xg:

   $xg = (1.12 \times 10^{-5})\ (r)\ (rpm)^2$

   r = radial distance in cm (from axis of rotation to the bottom of the tube)

   rpm = revolutions per minute
10. Immediately decant the supernatant of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds (be consistent between racks), and blot excess liquid from lip of tubes.
    NOTE: Invert tubes only one time. Pellets are fragile and slipping may occur.
11. Count all tubes in a gamma counter for 1 minute. Calculate the ng/mL of Rat Insulin in unknown samples using automated data reduction procedures (see Section IX).

VII. ASSAY PROCEDURE (continued)

Assay Procedure Flow Chart

Day One						Day Two		
Set-up	**Step 1**	**Steps 2 - 3**	**Step 4**	**Step 5**	**Step 6**	**Step 7**	**Step 8**	**Steps 9 - 11**
Tube Number	**Add Assay Buffer**	**Add Standards/ QC/ Samples**	**Add ^{125}I-Insulin Tracer**	**Add Insulin Antibody**	**Vortex, Cover, and Incubate 20-24 hrs at 4°C**	**Add Precipitating Reagent**	**Vortex, and Incubate 20 min. at 4°C**	**Centrifuge @ 4°C for 20 min., Decant and Count**
1,2	-	-	100 µL	-		-		
3,4	200 µL	-	100 µL	-		1.0 mL		
5,6	100 µL	-	100 µL	100 µL		1.0 mL		
7,8	-	100 µL of 0.156 ng/mL	100 µL	100 µL		1.0 mL		
9,10	-	100 µL of 0.313 ng/mL	100 µL	100 µL		1.0 mL		
11,12	-	100 µL of 0.625 ng/mL	100 µL	100 µL		1.0 mL		
13,14	-	100 µL of 1.25 ng/mL	100 µL	100 µL		1.0 mL		
15,16	-	100 µL of 2.5 ng/mL	100 µL	100 µL		1.0 mL		
17,18	-	100 µL of 5.0 ng/mL	100 µL	100 µL		1.0 mL		
19,20	-	100 µL of 10.0 ng/mL	100 µL	100 µL		1.0 mL		
21,22	-	100 µL of QC 1	100 µL	100 µL		1.0 mL		
23,24	-	100 µL of QC 2	100 µL	100 µL		1.0 mL		
25,26	-	100 µL of unknown	100 µL	100 µL		1.0 mL		
27-n	-	100 µL of unknown	100 µL	100 µL		1.0 mL		

IX. CALCULATIONS AND TRANSFORMATIONS

A. Explanation

The calculations for Rat Insulin can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data.

NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.

B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, Bo) (5-6), and all duplicate tubes for standards and samples to the end of the assay.

2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.

3. Calculate the percentage of tracer bound [(Total Binding Counts/Total Counts) X 100] This should be 35-50%.

4. Calculate the percentage of total binding (%B/Bo) for each standard and sample.

   %B/Bo = (Sample or Standard/Total Binding) X 100

5. Plot the % B/Bo for each standard on the y-axis and the known concentrations of the standards on the x-axis using log-log graph paper.

6. Construct the reference curve by joining the points with a smooth curve.

7. Determine the ng/mL of Rat Insulin in the unknown samples and controls by interpolation of the reference curve.

   NOTE: When sample volumes assayed differ from 100 µl, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50 µl of sample is used, then calculated data must be multiplied by 2).

8. To convert Rat Insulin from ng/mL to pM, multiply ng/mL by 175.

X. INTERPRETATION

A. Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range review results with the supervisor.

2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.

3. The limit of sensitivity for the Rat Insulin assay is 0.081 ng/mL + 2SD (100 µL sample size).

4. The limit of linearity for the Rat Insulin assay is 10 ng/mL (100 µL sample size). Any result greater than 10 ng/mL should be repeated on dilution using Assay Buffer as a diluent.

XI. NORMAL FASTING RANGE

0.5 – 2.0 ng/mL

XII. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Rat Insulin that can be detected by this assay is 0.081ng/mL + 2SD when using 100µL sample size.

B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation.

ED_{80} = 0.26 ± 0.04 ng/mL
ED_{50} = 1.1 ± 0.15 ng/mL
ED_{20} = 5.0 ± 0.75 ng/mL

C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

Rat Insulin I	100%
Rat Insulin II	100%
Human Insulin	100%
Human Proinsulin	69%
Porcine Insulin	100%
Sheep Insulin	100%
Hamster Insulin	100%
Mouse Insulin	100%
Rat C-Peptide	ND
Glucagon	ND
Somatostatin	ND
Pancreatic Polypeptide	ND
IGF-I	ND
Human IGF-I	ND
Human IGF-II	ND

ND-not detectable

D. Precision

Within and Between Assay Variation

Sample No.	Mean ng/mL	Within % CV	Between % CV
1	0.5	2.2	8.9
2	0.8	1.4	9.1
3	1.4	4.3	8.5
4	3.2	1.8	9.1
5	3.7	4.6	9.4

Within and between assay variations were performed on five Rat Serum samples containing varying concentrations of Rat Insulin. Data (mean and % CV) shown are from five duplicate determinations of each serum sample in five separate assays.

XII. ASSAY CHARACTERISTICS (continued)

E. Recovery

Spike & Recovery of Insulin in Rat Serum

Sample No.	Insulin Added ng/mL	Observed ng/mL	Expected ng/mL	% Recovery
1	0	0.4	-	-
2	0.5	0.8	0.9	89
3	1.0	1.3	1.4	93
4	2.0	2.2	2.4	91
5	5.0	4.6	5.4	85

Varying concentrations of Rat Insulin were added to five Rat Serum samples and the Insulin content was determined by RIA. Mean of the observed levels from five duplicate determinations in five separate assays are shown. Percent recovery was calculated on the observed vs. expected.

F. Linearity

Effect of Serum Dilution

Sample No.	Volume Sampled	Observed ng/mL	Expected ng/mL	% of Expected
1	100 µL	1.0	1.0	100
	75 µL	1.0		100
	50 µL	1.0		100
	25 µL	0.8		80
2	100 µL	2.1	2.1	100
	75 µL	2.1		100
	50 µL	2.0		95
	25 µL	1.7		81
3	100 µL	2.9	2.9	100
	75 µL	2.9		100
	50 µL	2.9		100
	25 µL	2.7		93
4	100 µL	4.8	4.8	100
	75 µL	4.7		98
	50 µL	4.7		98
	25 µL	4.5		94

Aliquots of pooled Rat Serum containing varying concentrations of Insulin were analyzed in the volumes indicated. Dilution factors of 1.0, 1.33, 2.0, and 4.0 representing 100 µL, 75 µL, 50 µL and 25 µL, respectively, were applied in calculating observed concentrations. Mean Insulin levels and percent of expected for five separate assays are shown.

XII. ASSAY CHARACTERISTICS (continued)

G. Example of Assay Results

This data is presented as an example only and should not be used in lieu of a standard curve prepared with each assay.

Tube #	ID	CPM	Ave CPM	Ave Net CPM	% B/Bo	ng/mL
1	Totals	17455	18428			
2	″	19400				
3	NSB	648	668			
4	″	687				
5	Bo	9183	9233	8565		
6	″	9282			--	
Standards						
7	0.156 ng/Ml	8255	8135	7467	0.872	
8		8014				
9	0.313 ng/Ml	6998	7045	6377	0.745	
10		7092				
11	0.625 ng/Ml	5832	5978	5310	0.620	
12		6123				
13	1.25 ng/Ml	4178	4202	3534	0.413	
14		4226				
15	2.5 ng/Ml	2986	2973	2305	0.269	
16		2959				
17	5.0 ng/Ml	2246	2235	1567	0.183	
18		2223				
19	10.0 ng/Ml	1562	1550	882	0.103	
20		1537				
Controls/Unknown						
21	QC 1	6801	6792	6124	0.715	0.39
22		6782				
23	QC 2	3898	3916	3248	0.379	1.52
24		3934				
25-n	Unknown					

XIII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control (QC) specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. These and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website emdmillipore.com using the catalog number as the keyword.

Recommended batch analysis decision using two controls (Westgard Rules)[4]:

1. When both controls are within ±2 SD. Decision: Approve batch and release analyte results.

2. When one control is outside ±2 SD and the second control is within ±2 SD. Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

Technician check of systems:
1. Check for calculation errors
2. Repeat standards and controls
3. Check reagent solutions
4. Check instrument

XIV. REPLACEMENT REAGENTS

Reagent	Cat #
^{125}I- Insulin (<5 μCi, 185 kBq)	9011
Label Hydrating Buffer (27mL)	LHB-P
Rat Insulin Standards (2 mL each)	8013-K
Insulin Antibody (26 mL)	1013-K
Precipitating Reagent (260 mL)	PR-UV
QC 1&2 (1 mL each)	6000-K
Assay Buffer (40 mL)	AB-P

XV. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.

XVI. REFERENCES

1. Morgan, C.R. and Lazarow, A. Immunoassay of Insulin: Two antibody system. Plasma Insulin Levels in Normal, Subdiabetic, and Diabetic rats. *Diabetes* 12:115-126, 1963.

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3. Feldman, H. and Rodbard, D. "Mathematical Theory of Radioimmunoassay", in:W.D. Odell and Doughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: J.B. Leppincott Company; pp158-203, 1971.

4. Westgard, J.O., et. al. A Multi-rule Shewhart Chart for Quality Control in Clinical Chemistry. *Clin Chem* 27:493-501, 1981.