Sensitive Rat Insulin

250 Tubes

Cat. # SRI-13K

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

I.   INTENDED USE

This Sensitive 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 0.0329 ng/mL + 2 SD can be easily achieved when using a 100 µL serum or plasma sample in a two-day, disequilibrium assay.
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 calibration or 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 Sensitive Rat Insulin assay utilizes ^{125}I-labeled Insulin and a rat 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, 1% RIA
Grade BSA
Quantity: 40 mL/vial
Preparation: Ready to use

B. Rat Insulin Antibody (Sensitive)
Guinea Pig anti-Rat Insulin Serum in Assay Buffer
Quantity: 26 mL/vial
Preparation: Ready to use

C. ^{125}I-Insulin (Sensitive)
^{125}I-Insulin Label, HPLC purified (specific activity 367 µCi/µg)
Lyophilized for stability. Freshly iodinated label contains <3 µCi (<111 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. Used to hydrate ^{125}I-Insulin
(Sensitive)
Quantity: 27 mL/vial
Preparation: Ready to use

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

F. Quality Controls 1 & 2 (Sensitive)
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 $\leq$ -20°C. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at $\leq$ -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 there from, 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 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 work station 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 (RSO).

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.

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label
[125] I-Insulin (Sensitive) Tracer	9011S	**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.
Sensitive Rat Insulin Antibody	1013S-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 - 3,000 xg. (Use of fixed-angle buckets are 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 $\leq$ -20°C. Avoid multiple (>5) freeze/thaw cycles.
4. Avoid using samples with gross hemolysis or lipemia.

VIII.   ASSAY PROCEDURE

Standard Preparation

Use care in opening the Standard vial.

Label five glass tubes 1, 2, 3, 4, and 5. Add 1.0 mL Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 1.0 mL of the 1 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.

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 $\leq$ -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 0.5 ng/mL	1.0 mL	1.0 mL of 1 ng/mL
2	1.0 mL of 0.25 ng/mL	1.0 mL	1.0 mL of 0.5 ng/mL
3	1.0 mL of 0.125 ng/mL	1.0 mL	1.0 mL of 0.25 ng/mL
4	1.0 mL of 0.0625 ng/mL	1.0 mL	1.0 mL of 0.125 ng/mL
5	1.0 mL of 0.0313 ng/mL	1.0 mL	1.0 mL of 0.0625 ng/mL

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

A. Assay Set-Up, First Day

1. Pipette 300 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4), 200 µL to reference (Bo) tubes (5-6), and 100 µL 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).
4. Pipette 100 µL of Sensitive Rat Insulin antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
5. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

B. Second Day

6. Pipette 100 µL of ^{125}I-Insulin (Sensitive) to all tubes.
7. Vortex, cover, and incubate overnight (20-24 hours) at 4°C.

VIII.   ASSAY PROCEDURE (continued)

C. Third Day

8. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).

9. Vortex and incubate 20 minutes at 4°C.

10. 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 = rotational velocity of the rotor

11. 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.

12. 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).

Assay Procedure Flow Chart

| Tube # | First Day | | | | Second Day | | Third Day | | |
	Step 1	Step 2-3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10-12
	Add Assay Buffer	Add Standard / QC Sample	Add Sensitive Rat Insulin Antibody	Vortex, Cover, and Incubate 20-24 hrs at 4°C	Add ^{125}I-Insulin (Sensitive) Tracer	Vortex, Cover, and Incubate 20-24 hrs at 4°C	Add Precipitating Reagent	Vortex, and Incubate 20 min. at 4°C	Centrifuge for 20 min., Decant, and Count pellets
1,2	----	----	----		100 μL		----		
3,4	300 μL	----	----		100 μL		1.0 mL		
5,6	200 μL	----	100 μL		100 μL		1.0 mL		
7,8	100 μL	100 μL of 0.0313 ng/mL	100 μL		100 μL		1.0 mL		
9,10	100 μL	100 μL of 0.0625 ng/mL	100 μL		100 μL		1.0 mL		
11,12	100 μL	100 μL of 0.125 ng/mL	100 μL		100 μL		1.0 mL		
13,14	100 μL	100 μL of 0.25 ng/mL	100 μL		100 μL		1.0 mL		
15,16	100 μL	100 μL of 0.5 ng/mL	100 μL		100 μL		1.0 mL		
17,18	100 μL	100 μL of 1.0 ng/mL	100 μL		100 μL		1.0 mL		
19,20	100 μL	100 μL of Sens. QC 1	100 μL		100 μL		1.0 mL		
21,22	100 μL	100 μL of Sens. QC 2	100 μL		100 μL		1.0 mL		
23,24	100 μL	100 μL of unknown	100 μL		100 μL		1.0 mL		
25-n	100 μL	100 μL of unknown	100 μL		100 μL		1.0 mL		

IX. CALCULATIONS AND TRANSFORMATIONS

A. Explanation

The calculations for Sensitive 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 concentration of the standard 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).]

X. INTERPRETATION

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 supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity for the Sensitive Rat Insulin assay is 0.0329 ng/mL + 2 SD (100 µL sample size).
4. The limit of linearity for the Sensitive Rat Insulin assay is 1.0 ng/mL (100 µL sample size). Any result greater than 1.0 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 0.0329 ng/mL + 2 SD when using a 100 μL sample size.

B. Performance

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

ED_{80} = 0.09 ± 0.01 ng/mL

ED_{50} = 0.22 ± 0.01 ng/mL

ED_{20} = 0.55 ± 0.01 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%
Porcine Insulin   100%
Sheep Insulin   100%
Hamster Insulin   100%
Mouse Insulin   100%
Rat C-Peptide   *
Glucagon   *
Somatostatin   *
Pancreatic Polypeptide   *
IGF-1   *
*-not detectable

D. Precision

Within and Between Assay Variation

Sample No.	Mean ng/mL	Within % CV	Between % CV
1	0.11	5.8	10.8
2	0.14	5.5	7.9
3	0.30	2.7	7.1
4	0.66	5.0	3.8

Within and between assay variations were performed on four 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 Rat Insulin in Rat Serum

Sample No.	Insulin Added ng/mL	Observed ng/mL	Expected ng/mL	% Recovery
1	0.00	0.11	-	-
2	0.05	0.14	0.16	88
3	0.10	0.19	0.21	90
4	0.20	0.30	0.31	97
5	0.50	0.66	0.61	108

Varying concentrations of Rat Insulin were added to four 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	100µL	0.26	0.26	100
	50µL	0.25		96
	25µL	0.27		104
	10µL	0.31		119
2	100µL	0.38	0.38	100
	50µL	0.34		87
	25µL	0.33		87
	10µL	0.36		95
3	100µL	0.50	0.50	100
	50µL	0.44		88
	25µL	0.43		86
	10µL	0.46		92
4	100µL	0.63	0.63	100
	50µL	0.55		87
	25µL	0.53		84
	10µL	0.60		95

Aliquots of pooled rat serum containing varying concentrations of Insulin were analyzed in the volumes indicated. Dilution factors of 1, 2, 4 and 10 representing 100 µL, 50 µL, 25 µL and 10 µL, respectively, were applied in calculating observed concentrations. Mean Insulin levels and percent of expected for five separate assays are shown.

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	Rat Insulin ng/mL
1	**Totals**	11006	11029			
2	″	11052		--	--	
3	**NSB**	331	338			
4	″	344		--	--	
5	**Bo**	5130	5244	4906		
6	″	5357			--	
Standards						
7	**0.0313 ng/mL**	5091	5057	4719	0.962	
8	"	5022				
9	**0.0625 ng/mL**	4693	4712	4374	0.891	
10	"	4730				
11	**0.125 ng/mL**	4162	4117	3779	0.770	
12	"	4071				
13	**0.25 ng/mL**	2924	2999	2661	0.542	
14	"	3074				
15	**0.5 ng/mL**	1737	1692	1354	0.276	
16	"	1647				
17	**1.0 ng/mL**	1147	1132	794	0.162	
18	"	1116				
Controls/Unknown						
19	**QC 1**	4641	4614	4276	0.872	0.077
20	"	4587				
21	**QC 2**	3223	3248	2910	0.593	0.219
22	″	3273				
23-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

Reagents	Cat #
^{125}I-Insulin (Sensitive) (<3 μCi, <111 kBq)	9011S
Label Hydrating Buffer (27 mL)	LHB-P
Sensitive Rat Insulin Standards (2 mL each)	8013S-K
Rat Insulin Antibody (Sensitive) (26 mL)	1013S-K
Precipitating Reagent (260 mL)	PR-UV
Quality Control 1 & 2 (Sensitive) (1 mL each)	6000S-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

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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; pp 158-203, 1971

4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin Chem* 27:493-501, 1981