Glucagon

250 Tubes

Cat. # GL-32K

GLUCAGON RIA KIT
250 TUBES (Cat. # GL-32K)

I.	Intended Use	2
II.	Principles Of Procedure	2
III.	Reagents Supplied	3
IV.	Storage and Stability	4
V.	Reagent Precautions	4
VI.	Materials Required But Not Provided	5
VII.	Specimen Collection And Storage	5
VIII.	Assay Procedure	6
IX.	Calculations	8
X.	Interpretation	8
XI.	Normal Fasting Range	8
XII.	Assay Characteristics	9
XIII.	Quality Controls	12
XIV.	Replacement Reagents	12
XV.	Ordering Information	13
XVI.	References	13

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GLUCAGON RIA KIT
250 TUBES (Cat. # GL-32K)

I. INTENDED USE

EMD Millipore's Glucagon Radioimmunoassay (RIA) Kit utilizes an antibody which is specific for pancreatic glucagon. Cross reactivity to oxyntomodulin, the primary gut glucagon, is less than 0.1%. Sensitivity of 15.625 pg/mL can easily be achieved when using a 100 µL serum or plasma sample in a two-day, disequilibrium assay (400 µL Total Volume).

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 Glucagon assay utilizes ^{125}I-labeled Glucagon and a Glucagon antiserum to determine the level of Glucagon 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. Glucagon Assay Buffer
0.2M Glycine, pH 8.8, 0.03M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA
Quantity: 40 mL/vial
Preparation: Ready to use

B. Glucagon Antibody
Guinea Pig anti-Glucagon Serum in Assay Buffer
Quantity: 26 mL/vial
Preparation: Ready to use

C. ^{125}I-Glucagon
^{125}I-Glucagon Label, HPLC purified (specific activity 603 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 set at room temperature for 30 minutes, with occasional gentle mixing.

D. Glucagon Label Hydrating Buffer
Assay Buffer containing Glycine as a carrier. Used to hydrate ^{125}I-Glucagon.
Quantity: 27 mL/vial
Preparation: Ready to use

E. Glucagon Standards
Glucagon in Assay Buffer at the following concentration:
250 pg/mL
Quantity: 3 mL/vial
Preparation: Ready to use

F. Quality Controls 1 & 2
Purified Recombinant Glucagon 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 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 (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, and 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.

B. Sodium Azide

Sodium Azide has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide 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

Full hazardous labels for the components of this kit:

Ingredient, Cat #		Full Label	
Glucagon Quality Controls 1 and 2	6000-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.
125I-Glucagon	9030		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
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 pipet 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 ≤ -20°C. Avoid multiple (> 5) freeze/thaw cycles.

4. Avoid using samples with gross hemolysis or lipemia.

5. Glucagon must be protected from proteolysis during assay procedures and sample storage. Collect blood samples in serum or plasma tubes containing 250 KIU Trasylol (Aprotinin) per mL of whole blood. This will result in a final concentration of approximately 500 KIU Trasylol per mL of serum or plasma. Aliquot and freeze at -20°C to -70°C.

VIII. ASSAY PROCEDURE

Standard Preparation

Use care in opening the Standard vial.

Label four glass tubes 1, 2, 3, and 4. Add 1.0 mL Assay Buffer to each of the four tubes. Prepare serial dilutions by adding 1.0 mL of the 250 pg/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.

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	125 pg/mL	1.0 mL	1.0 mL of 250 pg/mL
2	62.5 pg/mL	1.0 mL	1.0 mL of 125 pg/mL
3	31.25 pg/mL	1.0 mL	1.0 mL of 62.5pg/mL
4	15.625 pg/mL	1.0 mL	1.0 mL of 31.25 pg/mL

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

Day One

1. Pipette 300 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 200 µL of Assay Buffer in the Reference (Bo) tubes (5-6). Pipette 100 µL of Assay Buffer to tubes seven through the end of the assay.

2. Pipette 100 µL of Standards and Quality Controls in duplicate (see assay flow chart).

3. Pipette 100 µL of each sample in duplicate. (NOTE: Smaller volumes of sample may be used when Glucagon 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 Glucagon 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 4ºC.

Day Two

6. Hydrate the ^{125}I-Glucagon tracer with 27 mL of Label Hydrating Buffer. Gently mix. Pipette 100 µL of ^{125}I-Glucagon to all tubes.

7. Vortex, cover and incubate overnight (22-24 hours) at 4ºC.

VIII. ASSAY PROCEDURE (continued)

Day Three

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, at 4°C, for 20 minutes at 2,000-3,000 xg.

Note: If less than 2,000 xg is used, 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

11. Immediately decant supernatant from all centrifuged tubes except Total Count tubes (1-2). Drain tubes for 15-60 seconds (be consistent between racks), blot excess liquid from lip of tubes and count pellet using the gamma counter according to the manufacturer's instructions.

Assay Procedure Flow Chart

Day One					Day Two		Day Three	
Set-up	Step 1	Step 2&3	Step 4	Step 5	Step 6	Step 7	Step 8	Steps 9-11
Tube Number	Add Assay Buffer	Add Standard/QC Sample	Add Glucagon Antibody	**Vortex, Cover, and Incubate 20-24 hrs at 4°C**	Add I-125 Glucagon Tracer	**Vortex, Cover and Incubate 22-24 hrs at 4°C**	Add Precipitating Reagent	**Incubate 20 min. at 4°C, Centrifuge at 4°C for 20 min Decant and Count**
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 15.625 pg/mL	100 µL		100 µL		1.0 mL	
9,10	100 µL	100 µL of 31.25 pg/mL	100 µL		100 µL		1.0 mL	
11,12	100 µL	100 µL of 62.5 pg/mL	100 µL		100 µL		1.0 mL	
13,14	100 µL	100 µL of 125 pg/mL	100 µL		100 µL		1.0 mL	
15,16	100 µL	100 µL of 250 pg/mL	100 µL		100 µL		1.0 mL	
17,18	100 µL	100 µL of QC 1	100 µL		100 µL		1.0 mL	
19,20	100 µL	100 µL of QC 2	100 µL		100 µL		1.0 mL	
21,22	100 µL	100 µL of unknown	100 µL		100 µL		1.0 mL	

IX. CALCULATIONS

A. Explanation

The calculations for Glucagon 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 pg/mL of Glucagon 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

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 Glucagon assay is 18.453 pg/mL +2 SD (100 µL sample size).

4. The limit of linearity for the Glucagon assay is 250pg/mL (100 µL sample size). Any result greater than 250 pg/mL should be repeated on dilution using Assay Buffer as a diluent.

XI. NORMAL FASTING RANGE

50-150 pg/mL

XII. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Glucagon that can be detected by this assay is 18.453 pg/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} = 53 ± 4 pg/mL
ED_{50} = 144 ± 11 pg/mL
ED_{20} = 394 ± 31 pg/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.

Glucagon	100 %
Oxyntomodulin	< 0.1 %
Human Insulin	*
Human Proinsulin	*
Human C-Peptide	*
Somatostatin	*
Pancreatic Polypeptide	*

*-not detectable

D. Precision

Within and Between Assay Variation

Sample No.	Mean pg/mL	Within % CV	Between % CV
1	60	6.8	13.5
2	65	4.0	12.7
3	90	4.6	13.4
4	220	4.0	7.3

Within and between assay variation was performed on four samples containing varying concentrations of Glucagon. Data (mean and % CV) shown are from four determinations of each sample in six separate assays.

XII. ASSAY CHARACTERISTICS (continued)

E. Recovery

Spike & Recovery of Glucagon in Canine Plasma

Sample No.	Glucagon Added pg/mL	Observed pg/mL	Expected pg/mL	% Recovery
1	0	25	-	-
2	20	44	45	98%
3	50	73	75	97%
4	100	121	125	97%
5	200	215	225	96%

Varying concentrations of Glucagon were added to five Canine Plasma samples and the Glucagon 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.

XII. ASSAY CHARACTERISTICS (continued)

F. 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	pg/mL
1	Totals	15578	15834			
2	″	16090				
3	NSB	456	455			
4	″	453				
5	Bo	5768	5833	5378		
6	″	5897				
Standards						
7	15.625 pg/mL	5289	5225	4770	88.7	
8		5161				
9	31.25 pg/mL	4575	4537	4082	75.9	
10		4498				
11	62.5 pg/mL	3456	3454	2999	55.8	
12		3452				
13	125 pg/mL	2171	2160	1705	31.7	
14		2149				
15	250pg/mL	1271	1337	882	16.4	
16		1402				
Controls/Unknown						
21	QC 1	4385	4371	3916	72.8	56.14
22		4357				
23	QC 2	2949	2917	2462	45.8	132.23
24		2884				
25-n	Unknown					

XIII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control 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 at 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-Glucagon (<3 µCi, 111 kBq)	9030
Glucagon Label Hydrating Buffer (27mL)	LHB-G
Glucagon Standards (3 mL each)	8030-K
Glucagon Antibody (26 mL)	1032-K
Precipitating Reagent (260 mL)	PR-UV
Quality Control 1&2 (1 mL each)	6000-K
Glucagon Assay Buffer (40 mL)	AB-G

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