Glucagon Chemiluminescent

96-Well Plate

Cat. # EZGLU-30K, EZGLU-30BK

Glucagon Chemiluminescent ELISA Kit
96-Well Plate
(Cat. #EZGLU-30K, EZGLU-30BK)

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I. INTENDED USE

This ELISA kit is used for the non-radioactive quantification of intact glucagon level in human, rat, mouse, and porcine serum/plasma samples. One kit is sufficient to measure 38 unknown samples in duplicate. ***For Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based on: 1) capture of glucagon molecules in the plasma, after extraction and reconstitution, by a specific anti-glucagon IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies, 2) and the simultaneous binding of a second biotinylated antibody to glucagon, 3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash-away of free enzyme, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in a luminometer at ~425 nm in the presence of a chemiluminescent substrate. The enzyme activity is measured by the increased relative light units (RLU). Since the increase in RLU is directly proportional to the amount of captured glucagon in the unknown sample, the concentration of glucagon can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of glucagon.

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Microtiter Plate with 2 plate sealers*	EPDAGPW	---------	1 plate 2 sealers
Porcine Glucagon Standard	E8030-K	lyophilized	1 bottle
Porcine Glucagon Quality Controls 1 and 2	E6030-K	lyophilized	2 vials
Assay Buffer	EAB-GLU	25 mL	1 vial
10X HRP Wash Buffer Concentrate	EWB-HRP	50 mL	2 bottles
Glucagon Capture Antibody	E1030-C	1.2 mL	1 vial
Glucagon Detection Antibody	E1030-D	1.2 mL	1 vial
Enzyme Solution	EHRP-6	12 mL	1 vial
Substrate Solution A	ESS-A	6 mL	1 vial
Substrate Solution B	ESS-B	6 mL	1 vial
Mixing Bottle	---------	---------	1 bottle

***Note: Unused wells should be resealed in the foil pouch with the dessicant provided and stored at 2-8 °C.**

IV. STORAGE AND STABILITY

Recommended storage for kit components is 2-8°C.

All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

V. REAGENT PRECAUTIONS

Sodium Azide has been added to certain reagents as a preservative. Although the concentrations are low, 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 labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
Glucagon Quality Controls 1 & 2	E6030-K		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment
Human Glucagon Standard	E8030-K		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Substrate Solution B	ESS-B		**Danger.** Causes skin irritation. Causes serious eye irritation. May cause cancer. Obtain special instructions before use. IF ON SKIN: Wash with plenty of soap and water. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Get medical advice/ attention.
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

VI. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. Pipettes and pipette tips: 10 µL -20 µL or 20 µL -100 µL
2. Multi-channel Pipettes and pipette tips: 5 -50 µL and 50 -300 µL
3. Repeator pipette and tip to deliver 10 µL - 20 µL volume
4. Buffer and Reagent Reservoirs
5. Vortex Mixer
6. De-ionized Water
7. Centrifuge capable of spinning with 17,000xg
8. Luminiometer Plate Reader capable of measuring glow at ~425 nm
9. Orbital Microtiter Plate Shaker
10. Absorbent Paper or Cloth
11. Protease inhibitor Aprotinin for serum and plasma sample preparation
12. Reagent grade Acetonitrile for serum and plasma extraction
13. A sample drying equipment to dry extracted samples, such as SpeedVac Concentration, Lyophilizer, or others

VII. SAMPLE COLLECTION AND STORAGE

1. To prepare plasma sample, whole blood should be collected into Vacutainer® EDTA-plasma tubes. Immediately add Aprotinin to final concentration of 500 KIU/mL, mix well, and centrifuged at 2,000 to 3,000xg for 15 minutes at 4 ± 2°C.

2. If heparin is to be used as anti-coagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

3. To prepare serum samples, whole blood is directly drawn into a Vacutainer® serum tube that contains no anticoagulant. Immediately add Aprotinin to final concentration of 500 KIU/mL and mix well, and let blood clot at room temperature for 30 min.

4. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.

5. Transfer and store serum/plasma samples in separate tubes. Date and identify each sample.

6. Use freshly prepared samples for assay or store samples in aliquots at ≤ –20°C for later use. For longer storage, keep at -70°C to -80°C. Avoid repeated freeze/thaw cycles (>2).

7. Because of known vulnerability of glucagon to proteases actions, it is better to use plasma samples.

VIII. SAMPLE EXTRACTION PROCEDURES

The extraction method described in this protocol yields sample results comparable to other commonly used procedures such as C18-reverse phase column chromatography or utilizing Waters 96-well HLB Extraction Plate. Although this method is the least costly it is to the assay operators discretion to choose their favorite extraction methods.

All extraction procedures in this method are performed at ambient temperature.

Option A) when sample quantity is not limited to 300 µL:

1. Place 300 µL serum/plasma sample in a 1.5 mL plastic microcentrifuge tube, add 450 µL Acetonitrile, immediately cap the tube and vortex vigorously about 5 seconds.

2. Let tube stand at ambient temperature for 10~30 minutes, then centrifuge at 17,000xg for 5 minutes. [Note: There is no difference in the glucagon recovery rate between 10 and 30 min].

3. Carefully remove 600 µL supernatant and transfer to a clean tube or 96-deep well plate for drying.

4. Dry supernatant with available equipment, such as heat block under N2 stream, lyophilizer, SpeedVac Concentrator, etc. For quicker drying, use SpeedVac Concentrator with the appropriate rotor at a setting of two hour heating at 80^{o}C and return to ambient for another 2 hours.

5. Store sealed dried supernatant at 4^{o}C until commencement of assay.

Option B) when sample quantity is less than 300 µL:

1. Place 150 µL serum/plasma sample in a 1.5 mL plastic microcentrifuge tube, add 225 µL Acetonitrile, immediately cap the tube and vortex vigorously about 5 seconds.

2. Let tube stand at ambient temperature for 10~30 minutes, then centrifuge at 17,000xg for 5 minutes. [Note: There is no difference in the glucagon recovery rate between 10 and 30 min].

3. Carefully remove 300 µL supernatant and transfer to a clean tube or 96-deep well plate for drying.

4. Dry supernatant with available equipment, such as heat block under N2 stream, lyophilizer, SpeedVac Concentrator, etc. For quicker drying, use SpeedVac Concentrator with the appropriate rotor at a setting of two hour heating at 80^{o}C and return to ambient for another 2 hours.

5. Store sealed dried supernatant at 4^{o}C until commencement of assay.

IX. REAGENT PREPARATION

A. Glucagon Standard Preparation

1. Carefully open the bottle of lyophilized Glucagon Standard and reconstitute entire content in 2 mL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well. The concentration of glucagon will be 2 ng/mL.

2. Label six polypropylene microfuge tubes with the additional concentrations of standards to be prepared: 0.02 ng/mL, 0.05 ng/mL, 0.1 ng/mL, 0.2 ng/mL, 0.5 ng/mL and 1 ng/mL. Add Assay Buffer to each of the six tubes according to the volumes outlined in the chart below. Dilute the 2 ng/mL standard stock according to the chart below. Vortex each tube briefly to ensure complete mixing.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles (>2).

Concentration of Standards	Volume of 2 ng/mL Stock to Add	Volume of Assay Buffer to Add
0.02 ng/mL	0.010 mL	0.990 mL
0.05 ng/mL	0.025 mL	0.975 mL
0.1 ng/mL	0.050 mL	0.950 mL
0.2 ng/mL	0.100 mL	0.900 mL
0.5 ng/mL	0.250 mL	0.750 mL
1 ng/mL	0.500 mL	0.500 mL
2 ng/mL	----	----

B. Glucagon Quality Control 1 and 2 Preparation

Carefully open bottles of lyophilized Quality Control 1&2 and reconstitute entire content of each bottle with 0.5 mL distilled or de-ionized water and mix well as described above. Unused portions of Quality Control should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles (>2).

C. Preparation of Capture and Detection Antibody Mixture

Prior to use, combine the entire contents of glucagon Capture Antibody (1.2 mL) and glucagon Detection Antibody (1.2 mL), or at a 1:1 ratio, and invert to mix thoroughly. The mixture should be used within a working day and discarded afterwards.

D. Preparation of Substrate Solution

Prior to use, mix the entire content of Substrate Solution A (6 mL) and Substrate Solution B (6 mL), or at a 1:1 ratio in mixing bottle provided, and mix thoroughly. The working solution is stable for ~8 hours at room temperature in dark. **Avoid prolonged exposure to the sun or any other intense light source**. Short-term exposure to typical laboratory lighting will not harm the working solution. Any remaining working substrate solution should be discarded after use and should not be re-used.

X. GLUCAGON ELISA ASSAY PROCEDURE

Option A: For samples prepared following extraction procedure option A.

Pre-warm all reagents to room temperature immediately before setting up the assay**.** Hydrate dried plasma/serum extract with 60 µL Assay Buffer. Observe to ensure the hydration is complete. This represents a 4-fold increase in the glucagon concentration to the original sample.

1. Dilute the 10X concentrated HRP Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Fill each well with 300 µL diluted Wash Buffer. Decant Wash Buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add 30 µL Assay Buffer to Blank wells and 10 µL to the rest.

4. Add in duplicate 20 µL Glucagon Standards in the order of ascending concentrations to the appropriate wells.

5. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells.

6. Add sequentially 20 µL of the unknown samples in duplicate to the remaining wells**.**

7. Add 20 µL Antibody Mixture solution **(see Reagent Preparation section)** to each well with a repeater or a multichannel pipette.

8. Cover the plate with plate sealer and incubate at room temperature for 3 hours on an orbital micro-titer plate shaker set to rotate at moderate speed, about 400 to 500 rpm.

9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.

10. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

11. Transfer Enzyme Solution into a reagent reservoir. Using a multichannel pipette, add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker. While the plate is shaking, proceed to the next step to prepare substrate solution.

X. GLUCAGON ELISA ASSAY PROCEDURE (CONTINUED)

12. Prepare enough amount of working Substrate Solution **(see Reagent Preparation section)** by mixing Substrate Solutions A and B. Keep working substrate away from strong light.

13. At the end of 30 min incubation, remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.

14. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

15. Transfer working Substrate Solution into a reagent reservoir. Using a multichannel pipette, add 100 µL of the solution to each well, and shake on the plate shaker for 0.5 to 1 minute.

16. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. **Measure relative light units at ~425 nm in a luminometer plate reader** within 5 minutes **after adding the substrate solution if comparisons of standard curve signals between assays are important. Longer periods between adding the substrate and evaluating the plate may result in significantly decreased signal intensity. However, the calculated sample results will not be affected even if the reading time is delayed to 25 minutes after substrate addition.**

Assay Procedure (Option A) for Glucagon ELISA Kit (Cat. # EZGLU-30K)

	Step 1	Step 2	Step 3	Step 4-6	Step 7	Step 8-10	Step 11	Step 11-14	Step 15		Step 16
Well #	**Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.**	**Wash plate 3X with 300 µL diluted HRP Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.**	Assay Buffer	Standards/QCs/ Samples	Capture/ Detection Ab. Mixture	**Seal, Agitate, Incubate 3 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µL Wash Buffer.**	Working Substrate	**Seal, Agitate 0.5 – 1 minute.**	**Measure relative light units at ~425 nm in a luminometer plate reader within 5 minutes**
A1, B1			30 µL	--	20 µL		100 µL		100 µL		
C1, D1			10 µL	20 µL of 0.02 ng/mL Standard							
E1, F1			10 µL	20 µL of 0.05 ng/mL Standard							
G1, H1			10 µL	20 µL of 0.1 ng/mL Standard							
A2, B2			10 µL	20 µL of 0.2 ng/mL Standard							
C2, D2			10 µL	20 µL of 0.5 ng/mL Standard							
E2, F2			10 µL	20 µL of 1 ng/mL Standard							
G2, H2			10 µL	20 µL of 2 ng/mL Standard							
A3, B3			10 µL	20 µL of QC 1							
C3, D3			10 µL	20 µL of QC 2							
E3, F3			10 µL	20 µL of Sample 1							
G3, H3 Etc.			10 µL	20 µL of Sample 2							

X. GLUCAGON ELISA ASSAY PROCEDURE (continued)

Option B: For samples prepared following extraction procedure option B.

Pre-warm all reagents to room temperature immediately before setting up the assay**.** Hydrate dried plasma/serum extract with 30 µL Assay Buffer. Observe to ensure the hydration is complete. This represents a 4-fold increase in the glucagon concentration to the original sample.

1. Dilute the 10X concentrated HRP Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Fill each well with 300 µL diluted Wash Buffer. Decant Wash Buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add 30 µL Assay Buffer to Blank wells and 20 µL to the rest.

4. Add in duplicate 10 µL Glucagon Standards in the order of ascending concentrations to the appropriate wells.

5. Add in duplicate 10 µL QC1 and 10 µL QC2 to the appropriate wells.

6. Add sequentially 10 µL of the unknown samples in duplicate to the remaining wells**.**

7. Add 20 µL Antibody Mixture Solution **(see Reagent Preparation section)** to each well with a repeater pipette.

8. Cover the plate with plate sealer and incubate at 4°C for ~ 44 - 48 hours on an orbital micro-titer plate shaker set to rotate at moderate speed, about 400 to 500 rpm. [Note: the length of incubation may be shortened to overnight if the glucagon concentrations in the samples are high enough to allow it.]

9. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.

10. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

11. Transfer Enzyme Solution into a reagent reservoir. Using a multichannel pipette add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.

X. GLUCAGON ELISA ASSAY PROCEDURE (continued)

12. Prepare enough amount of working Substrate Solution **(see Reagent Preparation section)** by mixing Substrate Solutions A and B. Keep working substrate away from strong light.

13. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader.

14. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

15. Transfer working Substrate Solution into a reagent reservoir. Using a multichannel pipette add 100 µL of the solution to each well, and shake on the plate shaker for 0.5 to 1 minute.

16. Measure relative light units at ~425 nm in a luminometer plate reader within 5 minutes **after adding the substrate solution if comparisons of standard curve signals between assays are desired. Longer periods between adding the substrate and evaluating the plate may result in significantly decreased signal intensity. However, the calculated sample results will not be affected even if the reading time is delayed to 25 minutes after substrate addition.**

Assay Procedure (Option B) for Glucagon ELISA Kit (Cat. # EZGLU-30K)

	Step 1	Step 2	Step 3	Step 4-6	Step 7	Step 8-10	Step 11	Step 11-14	Step 15		Step 16
Well #	**Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.**	**Wash plate 3X with 300 µL diluted HRP Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.**	Assay Buffer	Standards/QCs/ Samples	Capture/ Detection Ab. Mixture	**Seal, Agitate, Incubate ~44 - 48 hours at 4°C. Wash 3X with 300 µL Wash Buffer.**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µlL Wash Buffer.**	Working Substrate	**Seal, Agitate 0.5 – 1 minute.**	**Measure relative light units at ~425 nm in a luminometer plate reader within 5 minutes**
A1, B1			30 µL	--	20 µL		100 µL		100 µL		
C1, D1			20 µL	10 µL of 0.02 ng/mL Standard							
E1, F1			20 µL	10 µL of 0.05 ng/mL Standard							
G1, H1			20 µL	10 µL of 0.1 ng/mL Standard							
A2, B2			20 µL	10 µL of 0.2 ng/mL Standard							
C2, D2			20 µL	10 µL of 0.5 ng/mL Standard							
E2, F2			20 µL	10 µL of 1 ng/mL Standard							
G2, H2			20 µL	10 µL of 2 ng/mL Standard							
A3, B3			20 µL	10 µL of QC 1							
C3, D3			20 µL	10 µL of QC 2							
E3, F3			20 µL	10 µL of Sample 1							
G3, H3 Etc.			20 µL	10 µL of Sample 2							

XI. MICROTITER PLATE ARRANGEMENT : This is the preferred arrangement to use with a luminometer that sweeps the wells horizontally. Arrange the duplicate wells vertically if the luminometer is programmed to sweep wells vertically.

Glucagon ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	QC1	QC1								
B	Standard 0.02 ng/mL	Standard 0.02 ng/mL	QC2	QC2								
C	Standard 0.05 ng/mL	Standard 0.05 ng/mL	Sample 1	Sample 1								
D	Standard 0.1 ng/mL	Standard 0.1 ng/mL	Sample 2	Sample 2								
E	Standard 0.2 ng/mL	Standard 0.2 ng/mL	Etc.	Etc.								
F	Standard 0.5 ng/mL	Standard 0.5 ng/mL	Etc.	Etc.								
G	Standard 1 ng/mL	Standard 1 ng/mL										
H	Standard 2 ng/mL	Standard 2 ng/mL										

XII. CALCULATIONS

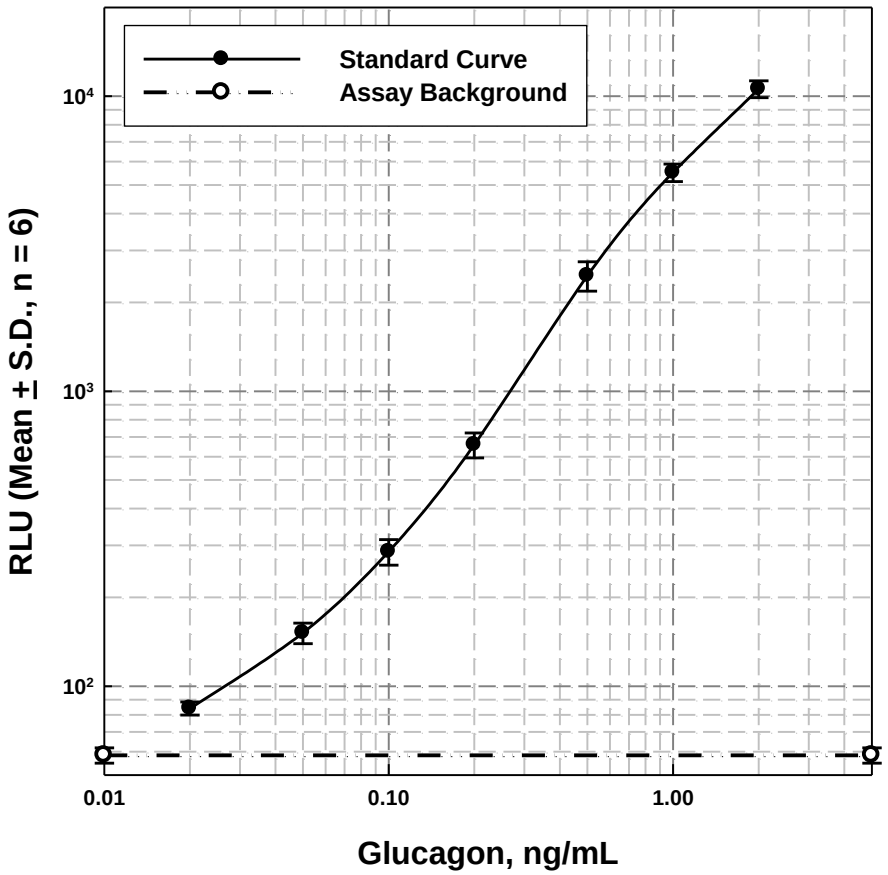
The dose-response curve of this assay fits best to a sigmoidal 4- or 5- parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function, then divided by the sample concentrating factor 4. If a different concentrating factor is adopted in the assay, use the appropriate factor for calculation.

Note: When sample volumes assayed differ from that specified in the assay protocol, an appropriate mathematical adjustment must be made to accommodate for the extra dilution factor. For example, in the case of assay protocol Option A, if 10 µL of sample is used instead of 20 µL, then calculated data must be multiplied by 2, then divided by 4. When sample volume assayed is less than specified in the protocol, compensate the volume deficit with Assay Buffer.

XIII. INTERPRETATION

1. The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.
3. The theoretical minimal detecting concentration of this assay is 0.003 ng/mL glucagon (20 µL 4X concentrated sample size).
4. The appropriate range of this assay is 0.02 ng/mL to 2 ng/mL glucagon in extracted samples. Any extracted sample with result greater than 2 ng/mL should be further diluted using Assay Buffer and the assay repeated until the results fall within range.

XIV. GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

XV. ASSAY CHARACTERISTICS

A. Analytical Sensitivity

The lowest level of glucagon that can be detected by this assay is 0.003 ng/mL in 20 µL extracted sample, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 6) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

B. Specificity

Glucagon (Human, Rat, Mouse, Porcine)	100.0%
Oxyntomodulin (Human, Rat, Mouse)	< 5%
Glucagon 1-18 (Human, Rat, Mouse, Porcine)	0%
Glucagon 19-29 (Human, Rat Mouse, Porcine)	0%

XV. ASSAY CHARACTERISTICS (continued)

C. Precision

Human Glucagon ELISA Precision: Summary of Assay Variations

Sample	I.D.	Intra-Assay Results		Inter-Assay Results	
		Mean ± S.D. (pg/mL)	CV	Mean ± S.D. (pg/mL)	CV
Serum	1	21.0 ± 0.83	3.91%	22.0± 1.32	6.00%
	2	134 ± 2.15	1.61%	132± 3.62	2.75%
Plasma	1	34.8 ± 1.07	3.09%	35.2 ± 1.08	3.06%
	2	167 ± 1.59	0.95%	164 ± 3.78	2.30%

Serum or plasma samples #1 and # 2 each are pooled from 4 donors and extracted for glucagon assay. #2 sample extracts are spiked with porcine glucagon before extraction. Assay variations are calculated from results of six independent assays.

D. Analyte Spike Recovery Rate

Sample ID	Glucagon Level				
	Basal	Spiked at Low Level*		Spiked at High Level*	
	pg/mL	pg/mL	Recovery	pg/mL	Recovery
1	15.8	55.8	90.1%	226.8	84.9%
2	11.5	51.5	90.1%	217.3	82.8%
3	25.5	67.8	96.0%	239.0	85.9%
4	59.0	101.0	95.5%	262.5	81.9%
5	50.0	91.0	93.2%	256.3	83.0%
Mean ± S.D. (n = 5)	----	----	93.0 ± 2.83%	----	83.7 ± 1.64%

***, Spiked glucagon levels before extraction: Low = 44 pg/mL, High = 248.5 pg/mL**

Human plasma samples are spiked with porcine glucagon, mixed, and extracted with 60% acetonitrile. Dried extracts are reconstituted in EAB-GLU equivalent to ¼ of original plasma volume and assayed for glucagon by ELISA.

E. Linearity of Sample Dilution

Sample I.D.	Glucagon Content Measured at Various Sample Volumes							
	20 µL		15 µL		10 µL		5 µL	
	pg/mL	% Expected	pg/mL	% Expected	pg/mL	% Expected	pg/mL	% Expected
1	1,903	100	1,433	100.4	942	99.0	472	99.1
2	1,876	100	1,410	100.2	942	100.4	473	100.8
3	1,914	100	1,399	97.5	957	100.0	469	97.9
4	1,840	100	1,440	104.3	948	103.0	492	107.0
Mean ± S.D.		100	--	100.6 ± 2.80	--	100.6 ± 1.70	--	101.2 ± 4.05

Four human plasma samples are spiked with porcine glucagon, mixed, and extracted with 60% acetonitrile. Dried extracts are reconstituted in EAB-GLU equivalent to ¼ of original plasma volume and assayed for glucagon by ELISA at indicated volumes.

XVI. QUALITY CONTROLS

The ranges for each 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.

XVII. TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results, the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay, the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started, all steps should be completed with precise timing and without interruption.
4. Avoid cross-contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. The intensity of light generated in the assay decays with time. If comparisons of assay signals between experiments are desired, measure light within 5 minutes after addition of substrate and make sure the luminometer is ready for use.
9. High signal in background or blank wells could be due to 1.) cross well contamination by standard solution or sample or 2.) inadequate washing of wells with Wash Buffer or 3.) overexposure to light after substrate has been added.

XVIII. REPLACEMENT REAGENTS

Reagents	Cat. #
Microtiter Plate	EPDAGPW
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Human Glucagon Standard	E8030-K
Quality Controls 1 and 2	E6030-K
Assay Buffer	EAB-GLU
Capture Antibody	E1030-C
Detection Antibody	E1030-D
Enzyme Solution	EHRP-6
Substrate Solution A	ESS-A
Substrate Solution B	ESS-B
10 - pack of Glucagon Chemiluminescent ELISA Kits	EZGLU-30BK

XIX. 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.