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User Guide

Glucagon Chemiluminescent ELISA Kit

96-Well Plate

EZGLU-30K
EZGLU-30BK

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Intended Use

This Glucagon Chemiluminescent 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.

This kit is for research use only. Not for use in diagnostic procedures.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- 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
- Simultaneous binding of a second biotinylated antibody to glucagon
- Wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies
- Wash-away of free enzyme
- 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.

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	**Volume**	**Quantity**	**Cat. No.**
Microtiter Plate with 2 plate sealers	-	1 plate 2 sealers	EPDAGPW
Porcine Glucagon Standard	Lyophilized	1 vial	E8030-K
Porcine Glucagon Quality Controls 1 and 2	Lyophilized	2 vials	E6030-K
Assay Buffer	25 mL	1 vial	EAB-GLU
10X HRP Wash Buffer Concentrate	50 mL/bottle	2 bottles	EWB-HRP
Glucagon Capture Antibody	1.2 mL	1 bottle	E1030-C
Glucagon Detection Antibody	1.2 mL	1 bottle	E1030-D
Enzyme Solution	12 mL	1 bottle	EHRP-6
Substrate Solution A	6 mL	1 bottle	ESS-A
Substrate Solution B	6 mL	1 bottle	ESS-B
Mixing Bottle	-	1 bottle	-

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

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.

Reagent Precautions

Sodium azide has been added to some 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.

Ingredient	Cat. No.	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.

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Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Repeater pipette and tip to deliver 10 µL-20 µL volume
- Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized Water
- Centrifuge capable of spinning with 17,000 x *g*
- Luminiometer Plate Reader capable of measuring glow at ~425 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth
- Protease inhibitor aprotinin for serum and plasma sample preparation
- Reagent grade Acetonitrile for serum and plasma extraction
- A sample drying equipment to dry extracted samples, such as SpeedVac™ Concentration, Lyophilizer, or others.

Sample Collection and Storage

Preparation of Serum Samples

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 centrifuge at 2,000 to 3,000 x *g* 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, 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 and −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.

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 operator’s discretion to choose their favorite extraction methods.

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

When sample quantity is not limited to 300 µL

Option A)

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 17,000 x *g* 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 °C and return to ambient for another 2 hours.
5. Store sealed dried supernatant at 4 °C until commencement of assay.

When sample quantity is less than 300 µL

Option B)

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 to 30 minutes, then centrifuge at 17,000 x *g* 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 °C and return to ambient for another 2 hours.
5. Store sealed dried supernatant at 4 °C until commencement of assay.

Reagent Preparation

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.

8. 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 reconstituted standard should be stored in small aliquots at ≤ −20 °C. Avoid multiple freeze/thaw cycles (> 2).

Tube #	Volume of Deionized Water to Add	Standard Stock Concentration
Reconstituted Standard	2 mL	2 ng/mL

Tube #	Volume of Assay Buffer to Add	Volume of 2 ng/mL Stock to Add	Standard Concentration
1	0.990 mL	0.010 mL	0.02 ng/mL
2	0.975 mL	0.025 mL	0.05 ng/mL
3	0.950 mL	0.050 mL	0.1 ng/mL
4	0.900 mL	0.100 mL	0.2 ng/mL
5	0.750 mL	0.250 mL	0.5 ng/mL
6	0.500 mL	0.500 mL	1.0 ng/mL

Glucagon Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each glucagon Quality Control 1 and Quality Control 2 with 0.5 mL distilled or de-ionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ −20 °C. Avoid further freeze/thaw cycles.

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), at a 1:1 ratio, and invert to mix thoroughly. The mixture should be used within a working day and discarded afterwards.

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.

Glucagon ELISA Assay Procedure

For samples prepared following extraction procedure Option A

Warm all reagents to room temperature before setting up the assay. Hydrate dried plasma/serum extract with 60 µL of 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.
12. Prepare enough amount of working Substrate Solution (see Reagent Preparation) 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

	Step 1	Step 2	Step 3	Step 4-6	Step 7	Step 8-10	Step 11	Step 11-14	Step 15		Step 16
Well #			Assay Buffer	Standards/ QCs/ Samples	Capture/ Detection Antibody		Enzyme Solution		Working Substrate		
A1, B1	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.	30 µL	--	20 µL ↓	Seal, Agitate, Incubate 3 hours at Room Temperature. Wash at 3X with 300 µL Wash Buffer.	100 µL ↓	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash at 6x with 300 µL Wash Buffer	100 µL ↓	Seal, Agitate 0.5–1 minute.	Measure relative units at ~425 nm in a luminometer plate reader within 5 minutes
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							

For samples prepared following extraction procedure Option B

Warm all reagents to room temperature before setting up the assay. Hydrate dried plasma/serum extract with 30 µL of 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) to each well with a repeater or a multichannel pipette.

8. Cover the plate with plate sealer and incubate at 4 °C for 44 to 48 hours on an orbital micro-titer plate shaker set to rotate at moderate speed, about 400 to 500 rpm. (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. While the plate is shaking, proceed to the next step to prepare substrate solution.

12. Prepare enough amount of working Substrate Solution (see Reagent Preparation) 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 B) for Glucagon ELISA Kit

	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 Antibody	Seal, Agitate, Incubate ~44–48 hours at 4 °C. Wash at 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 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	↓		↓		↓		

Microtiter Plate Arrangement

Glucagon ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	QC 1	QC 1								
B	Tube 1 0.02 ng/mL	Tube 1 0.02 ng/mL	QC 2	QC 2								
C	Tube 2 0.05 ng/mL	Tube 2 0.05 ng/mL	Sample 1	Sample 1								
D	Tube 3 0.10 ng/mL	Tube 3 0.10 ng/mL	Sample 2	Sample 2								
E	Tube 4 0.20 ng/mL	Tube 4 0.20 ng/mL	Etc.	Etc.								
F	Tube 5 0.50 ng/mL	Tube 5 0.50 ng/mL	Etc.	Etc.								
G	Tube 6 1.0 ng/mL	Tube 6 1.0 ng/mL										
H	Reconstituted Standard 2 ng/mL	Reconstituted Standard 2 ng/mL										

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Calculations

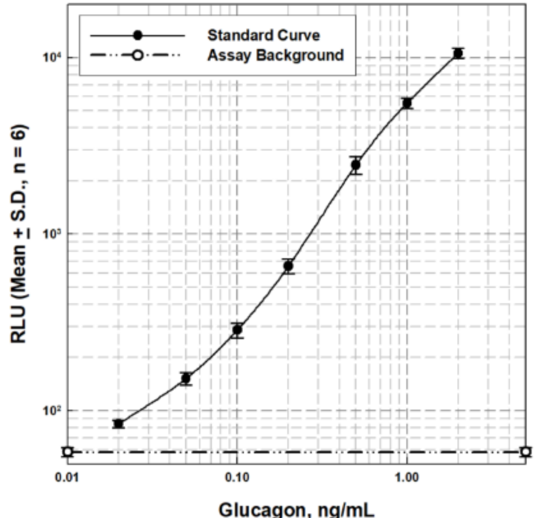
The dose-response curve of this assay fits best to a sigmoidal 4- or 5- parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function, then divided by the sample concentrating factor 4. If a different concentrating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

Interpretation

- 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.
- If the difference between duplicate results of a sample is > 15% CV, repeat the sample.
- The theoretical minimal detecting concentration of this assay is 0.003 ng/mL glucagon (20 µL 4X concentrated sample size).
- 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.

Graph of Typical Reference Curve



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

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Assay Characteristics

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.

Specificity

Glucagon (Human, Rat, Mouse, Porcine)	100%
Oxyntomodulin (Human, Rat, Mouse)	< 5%
Glucagon 1-18 (Human, Rat, Mouse, Porcine)	0%
Glucagon 19-29 (Human, Rat, Mouse, Porcine)	0%

Precision

Intra-Assay Variation

	Mean Human Glucagon Levels ±2 S.D. (pg/mL)	Intra-Assay %CV
Serum 1	21.0 ±0.83	3.91%
Serum 2	134 ±2.15	1.61%
Plasma 1	34.8 ±1.07	3.09%
Plasma 2	167 ±1.59	0.95%

Inter-Assay Variation

	Mean Human Glucagon Levels ±2 S.D. (pg/mL)	Inter-Assay %CV
Serum 1	22.0 ±1.32	6.00%
Serum 2	132 ±3.62	2.75%
Plasma 1	35.2 ±1.08	3.06%
Plasma 2	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.

Spike Recovery of Human Glucagon in Assay Samples

Sample	Basal (pg/mL)	At Low Level* (pg/mL)	Low Level Recovery	At High Level* (pg/mL)	High Level 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 ±1.67%

* 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.

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.

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Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- 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.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- 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.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- 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.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- 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.
- High signal in background or blank wells could be due to:
- cross well contamination by standard solution or sample, or
- inadequate washing of wells with Wash Buffer, or
- overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
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

Notice

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