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Glucagon ELISA

REF IB59142	Rx ONLY IVD
Effective Date: August 23, 2024	Version: USA-1.0

1. INTENDED PURPOSE & USE

For the quantitative measurement of Glucagon in human EDTA plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

Results shall be combined with other clinical and laboratory data to aid physicians in diagnosing carbohydrate metabolism disorders related to glucagon levels, including hyper- and hypoglycemia, and pancreatitis.

This kit is intended for professional use only and is for laboratory use only. For in vitro diagnostic use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for cancer screening or cancer diagnostic purposes.
2. This test is not intended to be used for diabetes management.
3. This test is not intended for home testing or self-testing.
4. The kit is calibrated for the determination of glucagon in human EDTA plasma. The kit is not calibrated for the determination of glucagon in other specimens of human or animal origin.
5. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
6. Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences.
7. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received a preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

3. SUPPLEMENTAL INFORMATION

Glucagon is a 29-amino acid peptide that is produced from the cleavage of proglucagon (160 amino acids) by proprotein convertase 2 in pancreatic alpha cells. In intestinal L-cells, proglucagon is cleaved into glicentin, a 69 amino acid peptide. Glicentin can further be processed into a 37 amino acid peptide oxyntomodulin. These peptides are released simultaneously upon stimulation [2-5].
Glucagon is involved in carbohydrate, fat, and protein metabolism and is the main catabolic hormone of the body. Glucagon raises the concentration of glucose and fatty acids in the bloodstream and plays an important role in maintaining glucose homeostasis by promoting gluconeogenesis and glycogenolysis. Glucagon has traditionally been considered an antagonist to insulin, with insulin reducing blood glucose levels and glucagon increasing them. As the level of blood glucose decreases, the pancreas releases more glucagon. As blood glucose increases, the pancreas releases less glucagon. Once blood glucose levels have normalized, glucagon secretion ceases [1, 2, 6, 7].

Glucagon also decreases fatty acid synthesis in adipose tissue and the liver and promotes lipolysis in these tissues, which causes them to release fatty acids into circulation where they can be catabolized to generate energy in tissues such as skeletal muscle when required [8]. Glucagon also regulates the rate of glucose production through lipolysis. Glucagon induces lipolysis in humans under conditions of insulin suppression [9].
Glucagon is secreted from the alpha cells of the pancreatic islets of Langerhans. Recent research has demonstrated that glucagon production may also occur outside the pancreas, with the gut being the most likely site of extrapancreatic glucagon synthesis [10].
Glucagon in diabetics has been found elevated absolutely or relatively to insulin, and it has been proposed that glucagon contributes to the development of hyper- and hypoglycemia [11-15]. Researchers have found that patients with diabetes are more likely to develop pancreatitis [16-20].

4. PRINCIPLE OF THE TEST

The Glucagon ELISA is a two-step capture or ‘sandwich’ type immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for glucagon is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of glucagon is conjugated to horse radish peroxidase (HRP conjugate). In the first incubation step, glucagon present in the specimen samples, calibrators and controls is bound to the antibody immobilized onto the microplate. Excess and unbound materials are removed by a washing step.
In the second incubation step, HRP conjugate antibody (HRP conjugate) is added, which binds specifically to any immobilized glucagon, thus forming a sandwich complex. Unbound HRP conjugate is removed by a washing step. Next, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue coloured product that is directly proportional to the amount of glucagon present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of glucagon in specimen samples and controls can be directly read.

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory *in vitro* use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
   - Wear protective clothing and disposable gloves.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. When adding deionized water for the reconstitution of lyophilized components, it is recommended to pre-wet the pipette tip to ensure an accurate transfer of water.
10. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label. For reconstituted lyophilized reagents, follow storage requirements in section *9. Reagents Provided.*
11. A calibrator curve must be established for every run.
12. It is recommended to all customers to prepare their own control materials or plasma pools which should be included in every run at a high and low level for assessing the reliability of results.
13. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
14. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
15. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
16. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
17. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
18. Plasma samples with a known glucagon concentration of less than 50 pg/mL may be used to dilute plasma samples with glucagon concentrations higher than 352 pg/mL. Otherwise, results may be reported as “> 352 pg/mL”. The use of any other reagent will lead to false results.
19. Avoid microbial contamination of reagents.
20. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
21. To prevent the contamination of reagents, do not pour reagents back into the original containers.
22. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
23. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
24. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
25. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
26. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
27. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
28. Do not reuse the microplate wells, they are for SINGLE USE only.
29. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
30. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

6. SAFETY CAUTIONS AND WARNINGS

6.1 BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrator stock and controls provided with the kit contain a material of human origin that has been found to be not infectious. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

6.2 CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

7. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

7.1 Specimen Collection & Storage

Follow the specimen collection procedure steps in the order provided below to avoid any delays that could potentially affect the stability of specimen samples. K2 and K3 EDTA collection tubes are suitable for plasma sample collection.

Approximately 0.3 mL of K2 or K3 EDTA plasma is required per duplicate determination.

1. Prior to sample collection, place a K2 or K3 EDTA plasma collection tube into a container of ice for at least 10 minutes.
2. Collect 4–5 mL of venous blood into an appropriately labelled pre-cooled EDTA plasma collection tube.
3. Mix the tube by inverting several times.
4. Place the collection tube into a container of ice to keep cool prior to centrifugation.
5. Centrifuge the tube at 2000x g for 10 minutes.
6. Immediately transfer the plasma into a labelled tube and store at ≤ -20°C. It is important to complete this step promptly to avoid sample degradation.
7. Plasma samples must be stored frozen at ≤ -20°C and are stable for up to 3 months. Avoid no more than 2 freeze/thaw cycles.

7.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

8. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 40 – 120 µL and 500 – 1000 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL and 100 µL.
3. Calibrated multi-channel pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Disposable pipette tips.
6. Distilled or deionized water.
7. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
8. Polypropylene or HDPE tubes for calibrator preparation (e.g., polypropylene microcentrifuge tubes).
9. Centrifuge capable of 2000x g.
10. Container with ice.
11. Vortex mixer.

9. REAGENTS PROVIDED

1. MPL Microplate

Contents:	One anti-glucagon monoclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.

2. HRP CONJ CONC HRP Conjugate Concentrate

Contents:	One bottle containing anti-glucagon monoclonal antibody-Horse Radish Peroxidase (HRP) conjugate in a stabilized buffer with a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	0.3 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months. Following Preparation: The HRP conjugate working solution is stable for 8 hours at room temperature following preparation.
Preparation of HRP Conjugate Working Solution:	**X101 Dilute 1:101 Before Use** Dilute 1:101 in Conjugate Diluent before use (e.g., 40 µL of HRP conjugate concentrate in 4 mL of conjugate diluent). If the whole plate is to be used dilute 120 µL of HRP conjugate concentrate in 12 mL of conjugate diluent.

3. CAL STK LYO Calibrator Stock Lyophilized

Contents:	One bottle of calibrator stock containing glucagon in a protein-based buffer with a non-mercury preservative. Used for the preparation of glucagon calibrators.
Format:	Lyophilized and Concentrated; Requires Preparation
Storage:	2–8°C (unopened)
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening and Reconstitution: Store remaining reconstituted Calibrator Stock at ≤ -20°C for up to 2 months with no more than 2 freeze/thaw cycles.
Reconstitution:	Reconstitute the lyophilized Calibrator Stock by adding 0.5 mL of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming before use. **Only reconstitute the Calibrator Stock immediately prior to the preparation of Glucagon Calibrators.**
Preparation of Calibrators:	See section *10. Preparation of Glucagon Calibrators.*

4. CONTROL 1 – 2 LYO Control 1 – 2 Lyophilized

Contents:	Two bottles of lyophilized control containing different glucagon concentrations. Protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of glucagon. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Lyophilized; Requires Preparation
Storage:	2–8°C (unopened)
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening and Reconstitution: Stable for **2 hours** at room temperature. For long-term storage, store at ≤ -20°C for up to 2 months with no more than 2 freeze/thaw cycles.
Reconstitution:	Reconstitute each bottle of control (Control 1 & Control 2) by adding 0.5 mL of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming.

5. CONJ DIL Conjugate Diluent

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative. Used for the preparation of the HRP Conjugate Working Solution.
Format:	Ready to Use
Volume:	15 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.

6. CAL DIL Calibrator Diluent

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative. Used for the preparation of the Glucagon Calibrators.
Format:	Ready to Use
Volume:	15 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.

7. TMB SUB TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	18 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.

8. STOP Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	8 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months.
Safety:	Warning Refer to product SDS.

9. WASH BUFF CONC Wash Buffer Concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for 3 months. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.
Preparation of Wash Buffer Working Solution:	**X10 Dilute 1:10 Before Use** Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

10. PREPARATION OF GLUCAGON CALIBRATORS

Materials Required:

- Calibrator Stock.
- Calibrator Diluent.
- Calibrated single-channel pipettes.
- 7 x polypropylene or HDPE tubes. (e.g. polypropylene microcentrifuge tubes).

Do not use glass test tubes; Glucagon may bind to glass which can alter the results.

Procedure:

An accurate preparation of the calibrators is essential to the performance of the test. Please follow good pipetting practices specific to the brand of pipettes being used.

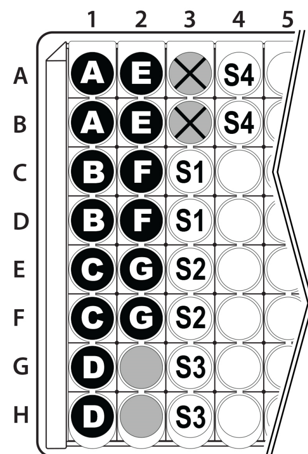
1. Label 7 x polypropylene or HDPE tubes as A, B, C, D, E, F & G, representing calibrators A-G.
2. Pipette 960 µL of Calibrator Diluent to tube G.
3. Pipette 500 µL of Calibrator Diluent to each tube A - F.
4. Reconstitute the Calibrator Stock as stated in section *9. Reagents Provided, 3. Calibrator Stock Lyophilized*.
5. Pipette 40 µL of reconstituted Calibrator Stock to tube G. Vortex the tube to mix thoroughly.
6. Immediately store the reconstituted Calibrator Stock at ≤ -20°C for future use.
7. Pipette 500 µL from tube G into tube F. Vortex tube F to mix thoroughly.
8. Pipette 500 µL from tube F into tube E. Vortex tube E to mix thoroughly.
9. Pipette 500 µL from tube E into tube D. Vortex tube D to mix thoroughly.
10. Pipette 500 µL from tube D into tube C. Vortex tube C to mix thoroughly.
11. Pipette 500 µL from tube C into tube B. Vortex tube B to mix thoroughly.

Use the prepared glucagon calibrators within 2 hours after preparation. Discard any leftover calibrators; do not store for future use.

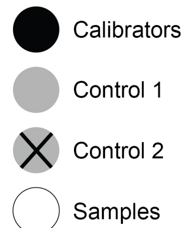
Preparation Summary Table

Calibrator	Glucagon (pg/mL)	Calibrator Diluent	Calibrator
Calibrator Stock Reconstituted	-	-	-
G	352	960 µL	40 µL of Calibrator Stock
F	176	500 µL	500 µL of G
E	88	500 µL	500 µL of F
D	44	500 µL	500 µL of E
C	22	500 µL	500 µL of D
B	11	500 µL	500 µL of C
A	0	500 µL	-

11. RECOMMENDED ASSAY LAYOUT



Legend



12. ASSAY PROCEDURE

⚠ **Follow the assay procedure steps in the order provided below to avoid any delays that could potentially affect the stability of components and specimen samples.**

All kit components must reach room temperature prior to use.
Thaw specimen samples at room temperature. Specimen samples should not be kept at room temperature for more than *30 minutes* prior to being tested. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components and specimen samples have reached room temperature, **mix** gently.
2. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section *11. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
3. **Prepare** the HRP Conjugate Working Solution and Wash Buffer Working Solution (See section *9. Reagents Provided, 2. HRP Conjugate Concentrate* and *9. Wash Buffer Concentrate*).
4. **Prepare** the Glucagon Calibrators and Controls (See section *10. Preparation of Glucagon Calibrators* and section *9. Reagents Provided, 3. Calibrator Stock Lyophilized, 4. Control 1-2 Lyophilized).*
5. **Pipette 100 µL** of each calibrator, control, and specimen sample into assigned wells.
6. **Incubate** the microplate at room temperature (no shaking) for **60 minutes**. Do not tap the microplate and avoid placing in intense light or air currents. Return specimen samples to frozen storage at ≤ -20°C.
7. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

   Automatic: Using an automatic microplate washer, perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

   Manually: Perform a **3-cycle** wash using **350 µL/well** of Wash Buffer Working Solution (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
8. **Pipette 100 µL** of the HRP Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended).
9. **Incubate** the microplate at room temperature (no shaking) for **30 minutes**. Do not tap the microplate and avoid placing in intense light or air currents.
10. **Wash** the microplate wells again as stated in step 7.
11. **Pipette 100 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).
12. **Incubate** the microplate at room temperature (no shaking) for **15 minutes**. Do not tap the microplate and avoid placing in intense light or air currents.
13. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
14. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

13. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 352 pg/mL and needs to be diluted and retested, then dilute it with an EDTA plasma sample that has a glucagon concentration of < 50 pg/mL. Do not dilute the sample more than 1:10. The result obtained must be multiplied by the dilution factor.

Sample Calculation:
EDTA sample #1 (high sample requiring dilution): >352 pg/mL
EDTA sample #2 (used to dilute sample #1): 20 pg/mL

EDTA sample #1 was diluted 1:10 using EDTA sample #2.
40 µL of EDTA sample #1 + 360 µL of EDTA sample #2.

1:10 diluted EDTA sample #1 was tested and had a glucagon concentration of 200 pg/mL.

Calculated concentration of EDTA sample#1:
= [(Conc. of 1:10 diluted EDTA sample #1) – (Conc. of EDTA sample #2) x % of total volume] x Dilution Factor

= (200 pg/mL –20 pg/mL x 90%) x 10
= 182 pg/mL x 10 = 1820 pg/mL

14. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the optical density acceptable range as stated in the QC Certificate.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

15. TYPICAL DATA

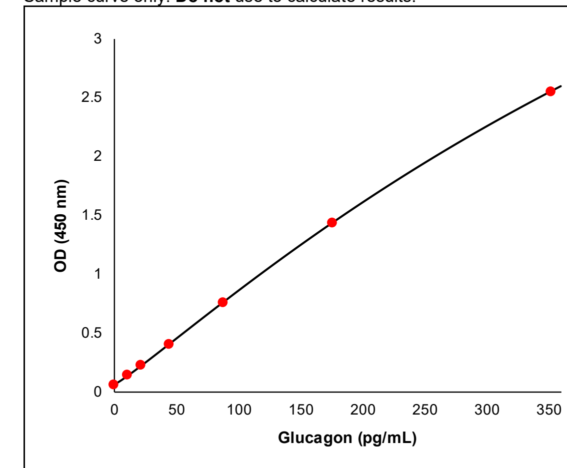
15.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	0.060	2	0
B	0.141	6	11
C	0.228	9	22
D	0.406	16	44
E	0.758	30	88
F	1.441	56	176
G	2.551	100	352
Unknown	0.430	-	47.1

15.2 TYPICAL CALIBRATOR CURVE

Sample curve only. **Do not** use to calculate results.



16. PERFORMANCE CHARACTERISTICS

16.1 SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline.
For the Limit of Blank (LoB), 96 replicates of each lot of Calibrator A (4 lots in total) across 6 tests (3 kit lots, 2 tests for each kit lot) were run.
For the Limit of Detection (LoD), 96 replicates of each low-value sample (4 samples in total) across 6 tests (3 kit lots, 2 tests for each kit lot) were run.
The Limit of Quantitation (LoQ) was estimated by determining the CV% of the glucagon concentration across a set of samples using three kit lots, 2 tests for each kit lot.
The results are summarized below.

Parameter	Glucagon
LoB	0.80 pg/mL
LoD	2.27 pg/mL
LoQ	5.33 pg/mL

16.2 SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with Glucagon (Glucagon (1-29)) cross-reacting at 100%.

Compound	% Cross-Reactivity
Glucagon (1-29)	100
Endothelin 1	0
GIP (1-42)	0
GIP (3-42)	0
Glicentin (By similarity) peptide	0
Glicentin-related polypeptide 1	0
GLP-1 (7-36)	0
GLP-1 (9-36)	0
GLP-2 (1-33)	0
Glucagon (3-29)	0
Oxyntomodulin	0
PACAP (1-27)	0
PACAP (1-38)	0.16
Pancreatic polypeptide	0
PHM 27	0
Secretin Peptide	0
Somatostatin	0
Substance P	0
VIP (10-28)	0
VIP (1-12)	0
VIP (1-28)	0
VIP (16-28)	0

16.3 INTERFERENCES

An interference study was performed according to the CLSI EP07-Ed3 guideline. Three human EDTA plasma samples were spiked with potentially interfering substances. No significant interference was detected up to the concentrations shown in the table below.

Compound	Test Concentration
Acetaminophen	15.6 mg/dL
Acetylcysteine	15 mg/dL
Acetylsalicylic Acid	3 mg/dL
Ampicillin Na	7.5 mg/dL
Bilirubin Conjugated	40 mg/dL
Bilirubin Unconjugated	40 mg/dL
Biotin	3.6 µg/mL
Captopril	1000 ng/mL
Captopril disulfide	10 µg/mL
Cefoxitin Na *	100 mg/dL
Cortisol	30 µg/dL
Cortisone	5 µg/dL
Cyclosporine	0.18 mg/dL
Doxycycline HCl	1.8 mg/dL
Enalaprilat dihydrate	200 ng/mL
Glucose	220 mg/dL
Haemoglobin	10 g/L
Heparin	3300 U/L
Human Anti-Mouse Antibodies (HAMAs)	1000 ng/mL
Ibuprofen	21.9 mg/dL
Insulin	150 µIU/mL
Levodopa	0.75 mg/dL
Loperamide	2 ng/mL
Metformin	12 µg/mL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Octreotide	7.8 ng/mL
Phenylbutazone	32.1 mg/dL
Prednisolone	1.2 µg/mL
Prednisone	99 ng/mL
Rheumatoid Factor **	50 IU/mL
Rifampicin	4.8 mg/dL
Semaglutide	150 ng/mL
Sulfathiazole sodium	150 µg/mL
Theophylline	6 mg/dL
Triglycerides	1500 mg/dL

* Cefoxitin Na higher than 100 mg/dL may cause an interference that leads to a decreased glucagon result. Under the advice of a physician, do not intake Cefoxitin Na during the overnight fasting period prior to blood collection.

** Rheumatoid factor levels higher than 50 IU/mL may cause an interference that leads to an increased glucagon result. Glucagon results from patients with rheumatoid arthritis (RA) should be interpreted with caution.

16.4 HIGH-DOSE HOOK EFFECT

No high-dose hook effect was observed for glucagon concentrations up to 2451 pg/mL.

16.5 PRECISION

The precision study was performed according to the CLSI EP05-A3 guideline.

Repeatability

The experimental protocol used a nested components-of-variance design with 20 testing days, two tests per day, and two replicates per run for each sample (a 20 x 2 x 2 design). The study included five EDTA plasma samples (S1-S5) and two kit controls (QC1 & QC2). The results were analyzed with a two-way nested ANOVA and are summarized in the table below. Mean and SD are reported in pg/mL.

Sample	Mean	Repeatability		Within-Laboratory Precision	
		SD	CV%	SD	CV%
QC1	42.8	0.86	2.0	1.44	3.4
QC2	230.2	2.60	1.1	7.32	3.2
S1	26.8	0.65	2.4	1.26	4.7
S2	54.7	1.20	2.2	3.09	5.6
S3	113.0	2.00	1.8	5.36	4.7
S4	238.2	4.57	1.9	13.10	5.5
S5	272.9	5.06	1.9	11.80	4.3

Reproducibility

The reproducibility across three different conditions was evaluated. The conditions were:

1. Lab 1: manually run.
2. Lab 2: manually run.
3. Lab 3: run with automated ELISA instrument.

The study used an experimental design model 3 x 5 x 5 (3 conditions x 5 testing days x 5 replicates per sample per day). The study included five EDTA plasma samples (S1-S5) and two kit controls (QC1 & QC2). The position of the kit controls and samples in the microplate was randomized from one day to another. Data was analyzed with a two-way nested ANOVA and are summarized in the table below. Mean and SD are reported in pg/mL.

Sample	Mean	Repeatability		Within-Location		Between-Location		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
QC1	40.9	1.11	2.7	2.98	7.3	0.00	0.0	2.98	7.3
QC2	220.0	5.26	2.4	13.12	6.0	0.00	0.0	13.12	6.0
S1	23.8	0.86	3.6	2.12	8.9	0.34	1.4	2.14	9.0
S2	48.5	1.36	2.8	2.94	6.1	3.33	6.9	4.44	9.2
S3	109.6	3.54	3.2	7.88	7.2	5.45	5.0	9.58	8.7
S4	219.5	6.85	3.1	19.33	8.8	8.10	3.7	20.96	9.5
S5	259.2	9.94	3.8	16.01	6.2	6.38	2.5	17.24	6.7

Lot-to-Lot Reproducibility

The reproducibility between three kit lots was evaluated by testing 3 kit lots with an experimental design model 3 x 5 x 5 (3 lots x 5 testing days x 5 replicates per sample per day). The study included five EDTA plasma samples (S1-S5). The position of the samples in the microplate was randomized from one day to another. Data was analyzed with a two-way nested ANOVA and are summarized in the table below. Mean and SD are reported in pg/mL.

Sample	Mean	Repeatability		Within-Lot		Between-Lot		Reproducibility	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
S1	26.3	0.79	3.0	1.42	5.4	2.77	10.5	3.11	11.8
S2	50.3	1.40	2.8	2.84	5.6	3.51	7.0	4.51	9.0
S3	108.0	3.00	2.8	6.14	5.7	3.53	3.3	7.08	6.6
S4	218.1	5.44	2.5	13.91	6.4	9.79	4.5	17.01	7.8
S5	260.6	7.04	2.7	10.38	4.0	4.13	1.6	11.18	4.3

16.6 LINEARITY

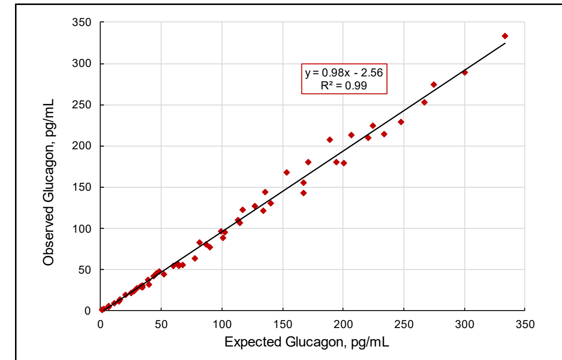
The linearity study was performed according to the CLSI EP06-Ed2 guideline using five EDTA plasma samples that covered the range of the assay.

The samples were diluted in EDTA plasma samples that were previously tested and had a glucagon concentration of < 50 pg/mL. The samples were diluted at several equidistant concentration levels, tested in quadruplicate, and the results were compared to the expected concentrations. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution when diluting samples in EDTA plasma samples with a glucagon concentration of < 50 pg/mL. Samples should not be diluted more than 1:10. The results (in pg/mL) are tabulated below.

Sample	Observed Result	Expected Result	Recovery %
1	127.31	-	-
1:2	55.03	64.98	84.7
1:5	24.38	27.59	88.4
1:10	12.04	15.12	79.6
Sample used for dilution = 2.7 pg/mL.			
2	274.57	-	-
1:2	130.20	140.47	92.7
1:5	55.07	60.02	91.8
1:10	30.47	33.20	91.8
Sample used for dilution = 6.4 pg/mL.			
3	48.30	-	-
1:2	22.18	24.97	88.8
1:5	9.59	10.98	87.3
1:10	5.49	6.31	87.0
Sample used for dilution = 1.6 pg/mL*.			
4	333.26	-	-
1:2	143.48	167.46	85.7
1:5	56.11	67.98	82.5
1:10	28.71	34.82	82.5
Sample used for dilution = 1.7 pg/mL*.			
5	224.80	-	-
1:2	144.57	135.38	106.8
1:5	83.15	81.73	101.7
1:10	56.99	63.85	89.3
Sample used for dilution = 46.0 pg/mL.			

* Estimated value.

Correlation graph of Observed vs. Expected results for all data points is shown below.



16.7 COMPARATIVE STUDIES

The IBL Glucagon ELISA kit (y) was compared against one competitor ELISA kit (x_1) and one immunoassay method (fluorescent + beads) (x_2) and yielded the following linear regression results.

$y = 0.97x_1 + 5.17$, $r = 0.995$, Slope =0.97, n=109.

$y = 0.94x_2 - 9.00$, $r = 0.97$, Slope =0.94, n=54.

17. REFERENCE RANGES

Reference ranges (95%) were estimated using EDTA plasma samples obtained from non-diabetic healthy adult individuals of diverse races. All donors were fasting for at least 8 hours before collecting samples. The samples were collected following the established procedure stated in section 7. Specimen Collection, Storage and Pre-Treatment.
Each laboratory shall establish its own reference ranges.

Group	N	Median (pg/mL)	Mean (pg/mL)	95% Confidence Range (pg/mL)
Healthy Non-diabetic Adults, Fasting	123	24.2	26.7	6.7 – 66.6

18. LITERATURE

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19. SYMBOLS GLOSSARY

Symbol	Definition	Symbol	Definition
REF	Catalogue number		Manufacturer
LOT	Batch code		Date of manufacture
IVD	In vitro diagnostic medical device		Biological risks
UDI	Unique Device Identifier		Consult instructions for use
X #	Dilute 1:# Before Use	Rx ONLY	Prescription only: Device restricted to use by or on the order of a physician
QTY	Quantity		Keep away from sunlight
	Use-by date	EC REP	Authorized representative in the European Community/ European Union
	Do not re-use		Temperature limit
	Caution	Σ	Contains sufficient for <n> tests
LYO	Lyophilized	RUO	For Research Use Only. Not for use in diagnostic procedures.

The definitions of symbols used for kit component names are described in the *Reagents Provided* section.

20. CHANGE HISTORY

Previous Version:	-	**New Version:**	-
Changes:	Build: *v1.5D* BASE: *v1.0*		

21. GENERAL INFORMATION

Manufactured For and Distributed By:	**Immuno-Biological Laboratories, Inc.** 8201 Central Ave. NE, Suite P Minneapolis, MN 55432, USA Phone: +1 (763)-780-2955 Email: info@ibl-america.com Web: www.ibl-america.com

Product Complaints

In the case of product complaints, the user shall submit in writing to the distributor or manufacturer a description of the complaint and provide accompanying data and/or information.

Warranty

IBL guarantees that the product is free of defects and will perform within the product specifications when the product is used prior to the expiration date, according to the intended purpose and use, and according to the instructions for use provided with the product. Any deviations from the intended purpose and use, instructions for use, modifications to kit components or use beyond the expiration date will invalidate any warranty claims.

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