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Glucagon ELISA Kit

03/21

(Catalog # E4907-100, 100 assays; Store at Multiple Temperatures)

I. Introduction:

Glucagon is a peptide hormone encoded by the *GCG* gene and is produced by alpha cells in the islets of langerhans of pancreas. It plays an important role in carbohydrate metabolism. When the glucose levels are low in the bloodstream, glucagon elevates the glucose level by promoting gluconeogenesis and glycogenolysis, which is in contrast to Insulin. However, glucagon and insulin work together to keep the blood glucose level stable. Abnormal glucagon levels are found in some pathological conditions such as glucagonoma, a rare tumor of the pancreatic alpha cells that results in excessive production of glucagon associated with mild diabetes, weight loss etc. Some diabetic patients have low glucagon levels too. Therefore, measuring glucagon levels is crucial for treatment and diagnostic purposes. **BioVision's Glucagon ELISA Kit** is a competitive ELISA kit for the quantitative determination of glucagon concentration in various biological samples such as serum and plasma. In this assay, glucagon-BSA conjugate is pre-coated on the 96-well plate. Glucagon in the sample and the antigen on the plate compete for binding to the anti-glucagon antibody. After the addition of the HRP conjugate that binds to captured glucagon antibody, TMB substrate is added and a color signal is developed. The intensity of color is inversely proportional to the amount of glucagon in the samples. This value is then compared to the glucagon standard curve and the glucagon concentration in sample(s) is calculated. The kit can detect as little as 10 ng/ml glucagon in less than 3 hr.

II. Application:

- *In vitro* quantitative determination of Glucagon

III. Sample Types:

- Human Serum or Plasma (Heparin or EDTA)

IV. Kit Contents:

Components	E4907-100	Cap Code	Part Number
Pre-Coated 96-Well Strip Plate	8 x 12 Strips	--	E4907-100-1
Glucagon Standard	20 µl	Yellow	E4907-100-2
Wash Buffer (10X)	40 ml	Blue/NM	E4907-100-3
Standard/Sample Diluent	50 ml	NM	E4907-100-4
Mouse Anti-Glucagon Antibody (1000X)	20 µl	Purple	E4907-100-5
HRP Conjugate (2500X)	20 µl	Blue	E4907-100-6
TMB Substrate	20 ml	Amber/NM	E4907-100-7
Stop Solution	20 ml	NM	E4907-100-8
Plate Sealers	2 films	--	E4907-100-9

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 650 nm and 450 nm)
- Precision multi-channel pipette and reagent reservoir
- Clean Eppendorf tubes

VI. Storage Conditions and Reagent Preparation:

Store the Standard/Sample Diluent, TMB Substrate and Stop Solution at 4 °C and rest of the kit components at -20 °C, protected from light. Briefly centrifuge all small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Prepare the working solutions, immediately prior to use.

- **Pre-Coated 96-well Strip Plate:** Do not open until ready to use. Bring to room temperature (RT) prior to opening. **After opening, immediately store the remaining unused Pre-coated strips at 4 °C in a foil bag with desiccant** to protect the strip wells from moisture.
- **Glucagon Standard (1 mg/ml):** Store at -20 °C.
- **Wash Buffer (10X):** Warm to RT prior to use. If crystals are present, mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by mixing 10 ml of Wash Buffer (10X) with 90 ml deionized water. 1X Wash Buffer is stable at 4 °C for one month.
- **Mouse Anti-Glucagon Antibody (1000X):** Dilute enough stock Mouse Anti-Glucagon Antibody (1000-fold) with Standard/Sample Diluent before use. Discard the diluted Anti-Glucagon Antibody after each use. Store the stock Anti-Glucagon Antibody at -20 °C.
- **HRP Conjugate (2500X):** Dilute the stock HRP Conjugate with 1X Wash Buffer to prepare 1X HRP Conjugate before use. Discard the diluted HRP Conjugate after each use. Store at -20 °C, protected from light. Avoid repeated freeze/thaw cycles.
- **Standard/Sample Diluent, TMB Substrate & Stop Solution:** Ready-to-use. Bring to RT prior to use. When not in use, reseal the bottles immediately and store at **4 °C**, protected from light.

VII. Glucagon ELISA Protocol:

1. Sample Preparation:

a. Collect serum or plasma samples using any standard methods. We recommend using either "off-the-clot" serum (collected in tubes, free of anticoagulants) or plasma collected using EDTA or lithium/sodium heparin. Once collected, serum/plasma samples may be stored at -20 °C for up to one month, prior to the assay.

b. To eliminate the matrix effect, spin filter samples using a 10 kDa Spin column (BioVision Cat # 1997-25 or similar) and collect ultrafiltrate. Add 50 µl of ultrafiltrate per sample well in duplicate. **Note:** Do not use serum/plasma that is hemolyzed or contaminated with red blood cells, as this may interfere with the assay.

2. Glucagon Standard Preparation:

a. Dilute 10 µl of stock Glucagon Standard (1 mg/ml) with 90 µl of Standard/Sample Diluent (1:10 dilution) to generate 100 µg/ml Glucagon Standard and mix well.

b. Mix 20 µl of 100 µg/ml Glucagon Standard with 980 µl Standard/Sample Diluent (1:50 dilution) to generate 2000 ng/ml Glucagon Standard (S7) and mix well. Prepare two-fold serial dilution (e.g. mix 300 µl Glucagon Standard with 300 µl of Standard/Sample diluent) to prepare Glucagon Standards S1-S6 (as shown below). S0 is the Standard/Sample Diluent. **Note:** A Standard Curve must be run with each assay.

Standards	S0	S1	S2	S3	S4	S5	S6	S7
Concentration (ng/ml)	0	31.25	62.5	125	250	500	1000	2000

3. Add 50 µl of Standard or test sample(s) to the designated wells.

4. Add 50 µl of 1X Mouse Anti-Glucagon Antibody to the Standard and Sample wells. Mix well and cover the plate with a plate sealer and incubate for 2 hr at 25 °C with gentle orbital shaking.

5. Remove the plate sealer and aspirate all reagents and wash each well 5 times. Wash by filling each well with 300 µl of 1X Wash Buffer to each well and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials. **Note:** A microplate washer can be used in this step and other wash steps. Complete removal of Wash Buffer is essential for accurate results.

6. Add 100 µl of 1X HRP Conjugate to each well. Mix well and cover with a plate sealer and incubate the plate for 60 min at 25 °C with gentle orbital shaking.

7. Aspirate all reagents and wash each well 5 times. Wash by filling each well with 300 µl of 1X Wash Buffer and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.

8. Add 100 µl of TMB Substrate to all wells. Tap or shake the plate occasionally to ensure complete mixing.

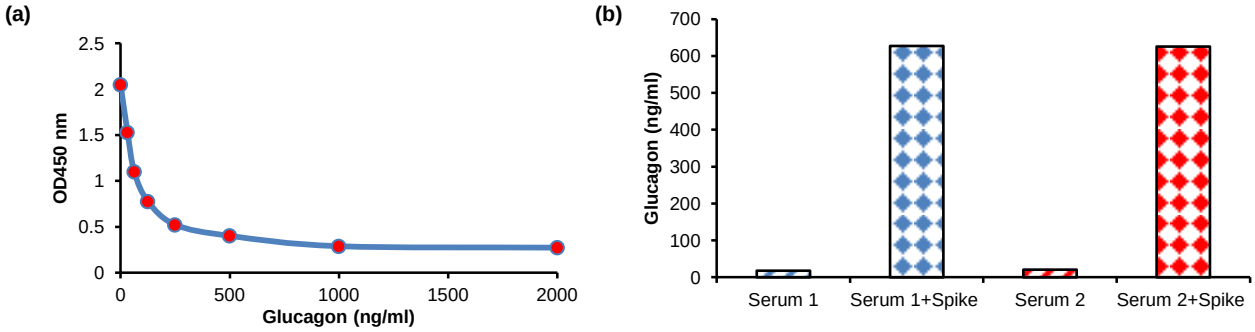
9. Measure the OD of the Glucagon Standard well (S0) at 650 nm. When the reading is ~0.8 (5-10 min), add 100 µl Stop Solution into each well and gently tap the plate to ensure thorough mixing.

10. Measure the OD of the plate at 450 nm at RT within 15 min.

Note: To minimize the variation in substrate incubation time, we recommend using a multichannel pipette and a reagent reservoir to add the TMB Substrate and the Stop Solution.

VIII. Calculation:

Plot the Glucagon Standard Curve as relative OD 450 nm of each Glucagon Standard (Y-axis) vs. the respective Glucagon concentrations (X-axis). The glucagon concentration in the test sample(s) is calculated from the Glucagon Standard Curve.



Figures. (a) Glucagon Standard Curve. *This Standard Curve is for demonstration only.* **(b)** Spike recovery experiment using human serum samples assayed with or without glucagon spike (500 ng/ml). Spike recovery was >95%.

VII. Related Products:

Insulin (mouse) ELISA Kit (K4271)
Folic Acid ELISA Kit (E4528)
Caffeine Acid ELISA Kit (E4558)
Bisphenol A ELISA Kit (E4722)
Ampicillin ELISA Kit (E4350)
Quinolone ELISA Kit (E4530)
Vancomycin ELISA Kit (E4605)
Carnosine ELISA Kit (E4766)

FOR RESEARCH USE ONLY! Not to be used on humans.