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GLP-1 (Human) ELISA Kit

rev 06/16

(Catalog # K4162-100, 100 assays, Store at 4°C)

I. Introduction:

Glucagon-like peptide-1 (GLP-1) is a neuropeptide and an incretin derived from the transcription product of the proglucagon gene. The major source of GLP-1 in the periphery is the intestinal L cell that secretes GLP-1 as a gut hormone; the major source in the brain is the nucleus of the solitary tract, which is the source of a widely distributed set of GLP-1 projection neurons. This kit was based on sandwich enzyme-linked immune-sorbent assay technology. The density of color is proportional to the Human GLP-1 amount of sample captured in plate.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of human GLP-1.
Detection Range: 0.313 - 20 ng/ml
Sensitivity: < 0.188 ng/ml

III. Specificity:

Human

IV. Sample Type:

Human serum, plasma, tissue homogenates and other biological fluids

V. Kit Contents:

Components	K4162-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4162-100-1
Lyophilized Standard	2 vials	K4162-100-2
Sample / Standard dilution buffer	20 ml	K4162-100-3
Biotin- detection antibody (Concentrated)	120 µl	K4162-100-4
Antibody dilution buffer	10 ml	K4162-100-5
HRP-Streptavidin Conjugate (SABC)	120 µl	K4162-100-6
SABC dilution buffer	10 ml	K4162-100-7
TMB substrate (Avoid light)	10 ml	K4162-100-8
Stop Solution	10 ml	K4162-100-9
Wash buffer (25X)	30 ml	K4162-100-10
Plate sealers	5	K4162-100-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

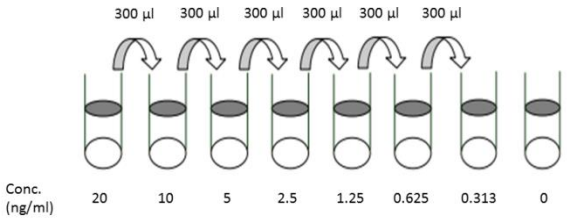
VII. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 month from the date of shipment. Avoid freeze-thaw cycles..

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer**: Dilute wash buffer at 1:25 with distilled water.
4. **Standard Preparation:**
   - Reconstitute the lyophilized Human GLP-1 standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 20 ng/ml standard stock solution.
   - Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Use within 2 hours of reconstituting.
   - Prepare 0.6 ml of 10 ng/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
   - Suggested standard points are: 20, 10, 5, 2.5, 1.25, 0.625, 0.313 ng/ml



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

5. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall into the optimal detection range of the kit.

- **Serum**: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000×g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.
- **Plasma**: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Analyze immediately or aliquot and store frozen at -20°C.
- **Tissue homogenates**: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to retrieve the supernatant.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.
Do not let wells completely dry at any time.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 100 µl of each **standard** and **samples** into appropriate wells. Cover well and incubate for 1.5 hours at 37ºC.
4. Remove the cover and discard the plate content without washing or letting the wells completely dry.
5. Add 0.1 ml of **Biotin-detection antibody** work solution into the above wells. Seal the plate and incubate at 37ºC for 60 min.
6. Discard the solution and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent material.
7. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
8. Discard the solution and wash 5 times with **1X Wash Solution** as step 6.
9. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 15-30 min. The shades of blue should be seen in the first 3-4 wells by the end of incubation.
10. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

X. CALCULATION:

For calculation, **(the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well).** The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human GLP-1 concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

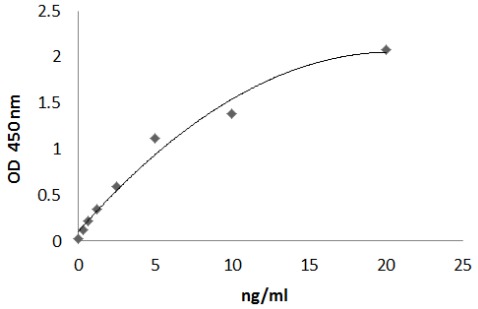


Figure: Typical Standard Curve: These standard curves are for demonstration only. (R^2= 0.982) A standard curve must be run with each assay.

XI. RELATED PRODUCTS:

- GLP-1 Antibody (Clone HGL-B5) (Cat. No. 3104-100)
- GLP-1, Human Recombinant (Cat. No. 7839-200, -1000)