RayBiotech
Empowering your proteomics

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Total GLP-1 ELISA Kit

Catalog Number: EIA-TGLP1

Manual
Last Revised: 06/02/2025

Introduction

Glucagon-like peptide-1 (GLP-1) is a highly conserved amino-acid-long peptide hormone among mammals and mainly secreted from the intestinal L-cells and the CNS. Functionally, GLP-1 plays an important role in energy homeostasis and nutrient absorption. The initial product GLP-1 (1–37) is susceptible to amidation and proteolytic cleavage, which gives rise to the two truncated and equipotent biologically active forms, GLP-1 (7–36) amide and GLP-1 (7–37). Then, the active forms of GLP-1 are extremely susceptible to the catalytic activity of the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4), resulting in the abundant inactivated GLP-1 (9–36) amide constituting 60–80% of total GLP-1 in circulation. This kit measures total GLP-1 including full length GLP-1 (1–37), the active (7- 36) and inactive (9-36) forms of GLP-1.

The RayBio® total GLP-1 Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting GLP-1 peptide based on the competitive ELISA principle. In this assay, a biotinylated GLP-1 peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated GLP-1 peptide competes with endogenous (unlabeled) GLP-1 for binding to the anti-GLP-1 antibody. After a wash step, any bound biotinylated GLP-1 then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated GLP-1 peptide and inversely proportional to the amount of endogenous GLP-1 in the standard or samples. A standard curve of known concentration of GLP-1 peptide can be established and the concentration of GLP-1 peptide in the samples can be calculated accordingly.

Storage

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see the Reagents table below.

Reagents

Component	Size / Description	Storage / Stability After Preparation
Microplate	96 wells (12 strips x 8 wells) coated with secondary antibody	1 month at 4°C*
Wash Buffer	25 mL of 20X concentrated solution	1 month at 4°C
Standard GLP-1 Peptide	2 vials of GLP-1 Peptide. 1 vial is enough to run each standard in duplicate.	The first standard: 2-3 days at 4°C. Additional dilutions: Do not store.
GLP-1 Antibody	2 vials of anti-GLP-1 Antibody	1 month at 4°C
Assay Diluent B	15 mL of 5X concentrated buffer.	1 month at 4°C
Biotinylated GLP-1 Peptide (Item F)	2 vials of Biotinylated GLP-1 Peptide. 1 vial is enough to assay the whole plate.	2-3 days at 4°C
HRP-Streptavidin	600 µL 250X concentrated HRP-conjugated streptavidin.	Do not store and reuse
Positive Control	1 vial of Positive Control	2-3 days at 4°C
TMB One-Step Substrate Reagent	12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	N/A
Stop Solution	8 mL of 0.2 M sulfuric acid	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional Materials Required

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1-liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- SigmaPlot software (or other software which can perform four-parameter logistic regression models)
- Tubes to prepare standard or sample dilutions
- Orbital shaker
- Aluminum foil
- Plastic wrap

Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

A. Preparation of Plate and Anti-GLP-1 Antibody

1. Equilibrate the plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the anti-GLP-1 antibody vial. Then add 50 µL of 1X Assay Diluent B to the vial to prepare the antibody concentrate. Pipette up and down to mix gently.
5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your anti-GLP-1 antibody working stock, which will be used in step 2 of Assay Procedure.

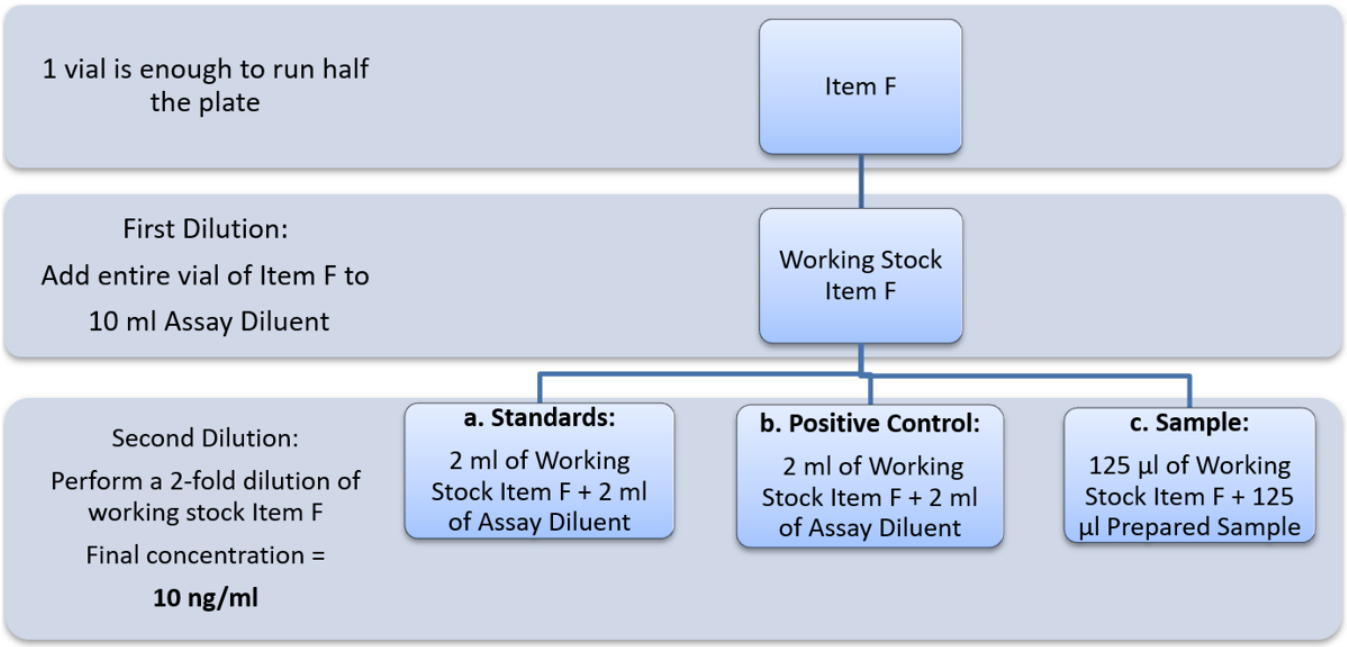
Note: The following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure)

B. Preparation of Biotinylated GLP-1 Peptide

6. Briefly centrifuge the vial of Biotinylated GLP-1 Peptide (Item F) before use.
7. See the image below for proper preparation of the Biotinylated GLP-1 Peptide. Transfer the entire contents of the biotinylated peptide vial into a tube containing 10 mL of 1X Assay Diluent B. This is your Biotinylated GLP-1 **working stock**. Pipette up and down to mix gently. *The final concentration of biotinylated GLP-1 will be **20 pg/mL.***

   a. Second Dilution of Biotinylated GLP-1 Peptide for Standards and Positive Control: Add 2 mL of working stock biotinylated peptide to 2 mL of 1X Assay Diluent B. The final concentration of this second dilution of biotinylated GLP-1 will be **10 pg/mL**. This will be your biotinylated GLP-1 working solution for preparing the standards and positive control.

   b. Second Dilution of Biotinylated GLP-1 Peptide for samples: Add 125 µL of working stock biotinylated peptide to 125 µL of prepared sample (see Sample Preparation section). This is a 2-fold dilution of your sample. The final concentration of biotinylated GLP-1 will be **10 pg/mL**.



C. Preparation of Standards

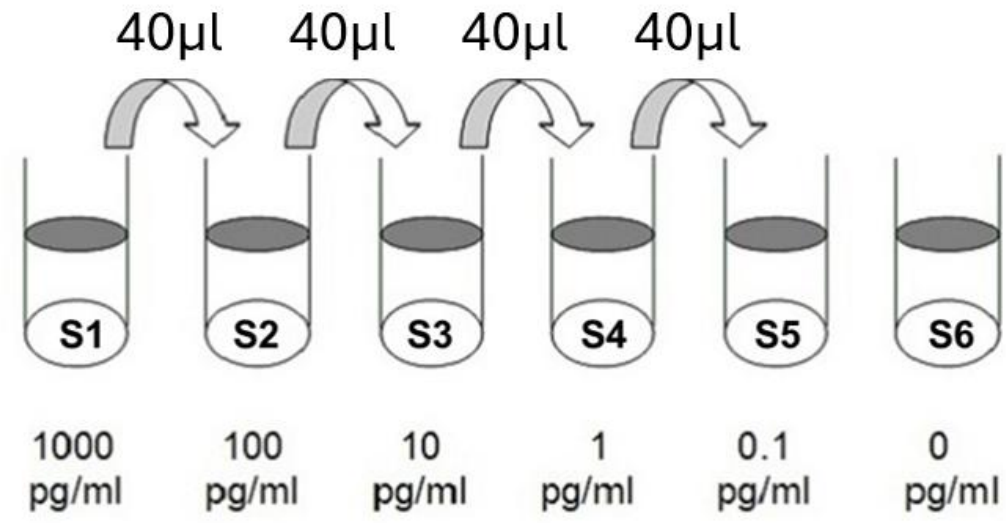
8. Briefly centrifuge the vial of GLP-1 Standard, pipette 400 µL of biotinylated GLP-1 working solution (**10 pg/mL,** prepared in step 7a) into the tube, and label the tube as S1 (1000 pg/mL). Mix thoroughly.

9. Label five additional microtubes with the following concentrations: S2 (100 pg/mL), S3 (10 pg/mL), S4 (1 pg/mL), S5 (0.1 pg/mL), and S6 (0 ng/mL). Pipette 360 µL of the biotinylated GLP-1 peptide working solution (**10 pg/mL,** prepared in step 7a) into each tube.

   It is very important to make sure the concentration of biotinylated GLP-1 is 10 pg/mL in all standards.

10. Pipette 40 µL of S1 into the tube labeled S2. Mix thoroughly.

11. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, add 40 µL of prior concentration to 360 µL of biotinylated GLP-1 until S5 (0.1 pg/mL) is reached. Mix each tube thoroughly before the next transfer.



D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial.

13. Add 400 µL of the biotinylated GLP-1 working solution (**10 pg/mL,** prepared in step 7a) to the vial of Positive Control. Mix thoroughly.

Positive Control is a cell culture media sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations, if no positive competition is observed please contact RayBiotech Technical Support. Positive Control may be diluted further if desired but be sure the final concentration of biotinylated GLP-1 is 10 pg/mL.

E. Sample Preparation

Note: Not compatible with plasma samples.

14. If you wish to perform a 2-fold dilution of your sample, proceed to step 7b. If you wish to perform a higher dilution of your sample, dilute your sample with 1X Assay Diluent B before performing step 7b.

    Example (to make a 4-fold dilution of sample):

    a. Dilute sample 2-fold (62.5 µL of sample + 62.5 µL of 1X Assay Diluent B).
    b. Perform step 7b (125 µL of working stock Biotinylated GLP-1 Peptide + 125 µL of sample prepared above).

    The total volume is 250 µL, enough for duplicate wells on the microplate.

    It is very important to make sure the final concentration of the biotinylated GLP-1 is **10 pg/mL**.

    Note: Optimal sample dilution factors should be determined empirically, however you may reference the following for recommended dilution factors for serum: **Human: 2X / Mouse: 2X / Rat: 2X.**

    If you have any questions regarding the recommended dilutions, you may contact technical support at 770-729-2992 or techsupport@raybiotech.com

Preparation of Wash Buffer and HRP

15. If the Wash Buffer contains visible crystals, warm to room temperature and mix gently until dissolved.

16. Dilute 20 mL of Wash Buffer concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

17. Briefly centrifuge the HRP-Streptavidin vial before use.

18. Dilute the HRP-Streptavidin concentrate 250-fold with 1X Assay Diluent B.

Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of Anti-GLP-1 Antibody (see Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with gentle shaking (1-2 cycle/sec). You may also incubate overnight at 4°C.
3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 µL each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of each standard (see Preparation of Standards section), Positive Control (see Positive Control Preparation section) and sample (see Sample Preparation section) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 1 hour at room temperature with gentle shaking (1-2 cycles/sec) overnight or at 4°C.
5. Discard the solution and wash 4 times as directed in step 3.
6. Add 100 µL of prepared HRP-Streptavidin solution (see Reagent Preparation step 18) to each well. Incubate for 45 minutes at room temperature with gentle shaking. It is recommended that the incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL anti-GLP-1 to each well. Incubate 1 hour at room temperature or overnight at 4°C.
3. Add 100 µL standard or sample to each well. Incubate 1 hour at room temperature or overnight at 4°C.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

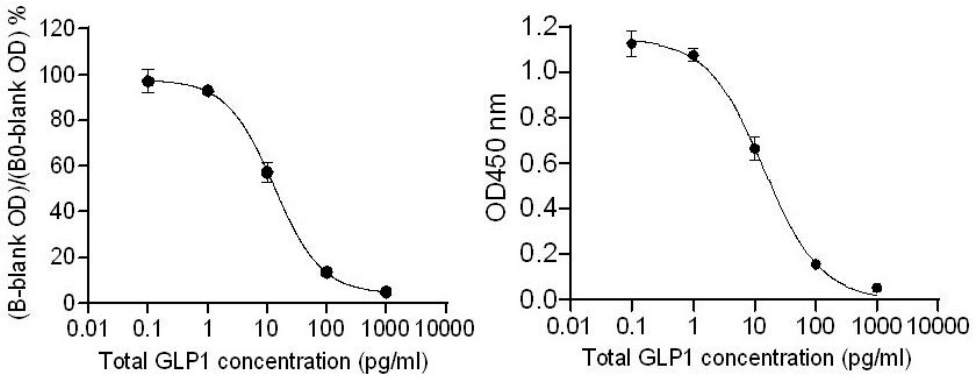
Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four- parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = (B-blank OD)/(B_0-blank OD) where:

- B = OD of sample or standard and
- B_0 = OD of zero standard (total binding)

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity

The minimum detectable concentration of total GLP-1 is 0.288 pg/ml.

Detection Range

0.1-1000 pg/mL

Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<15%

Specificity

This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Standard GLP-1 Peptide and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time; assay procedure step 2 may be done overnight • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Follow storage recomendations in manual. Keep substrate solution protected from light. • Add stop solution to each well before reading plate