Technical Manual

Human GLP-1 PharmaGenie ELISA Kit

- Catalogue Code: SBRS0027
- Competitive ELISA Principle
- Research Use Only

Contents

Key features and Sample Types

Aliases:

Glucagon [Cleaved into: Glicentin Glicentin-related polypeptide (GRPP) Oxyntomodulin (OXM) (OXY) Glucagon Glucagon-like peptide 1 (GLP-1) (Incretin hormone) Glucagon-like peptide 1(7-37) (GLP-1(7-37)) Glucagon-like peptide 1(7-36) (GLP-1(7-36)) Glucagon-like peptide 2 (GLP-2)]

Gene ID:

2641

Uniprot:

P01275

Detection method:

Competitive (Quantitative)

Range:

0.1-1000 pg/ml

Sensitivity:

0.1pg/ml

Sample Types:

Cell Culture Supernatants, Serum

Reactivity:

Human, Mouse, Rat

Recommended Dilutions:

Human: 2X
Mouse: 2X
Rat: 2X

Storage & Expiry

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 table below.

Introduction

Glucagon-like peptide-1 (GLP-1) is a 31 amino acid peptide hormone derived from selective cleavage of the proglucagon gene. It is mainly produced from enteroendocrine L-cells in GI tract. The other cleavage products derived from proglucagon genes are glucagon, GLP-2 and other small fragment peptides including Glicentin, Oxyntomodulin and two intervening peptides (IP-1 and IP-2).

Except for glucagon cleaved in alpha cells of the pancreas, all other cleaved peptides occurred in enteroendocrine L cells of intestine. GLP-1 has shown important roles in regulating glucose metabolic functions in humans. There are studies showing that GLP-1 is a potent anti-hyperglycemic hormone inducing glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion. This dual control of insulin and glucagon has the benefit that the plasma glucose concentration is kept in the normal fasting range to avoid hypoglycemia caused by overstimulation of insulin. In addition, GLP-1 was reported to restore the glucose sensitivity of pancreatic beta cells, probably via the upregualtion of GLUT2 and glucokinase. GLP-1 was reported to inhibit pancreatic beta-cell apoptosis and stimulate the proliferation and differentiation of insulinsecreting beta-cells.

GLP-1 has shown potential clinical application as a biomarker and treatment option for Diabetes Mellitus, which is based on the following physiological functions mediated by GLP-1.

- Regulating insulin secretion by increasing insulin secretion from the pancreas in a glucose-dependent manner and increasing insulin-sensitivity in both alpha and beta cells
- Regulating glucagon secretion by decreasing glucagon secretion from the pancreas by engagement of a specific G protein-coupled receptor.
- Regulating food intake by inhibiting acid secretion and gastric emptying in the stomach and decreasing food intake by increasing satiety in brain.

How do our ELISA kits work?

The Assay Genie GLP-1 Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting GLP-1 peptide based on the competitive enzyme immunoassay 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 GLP1 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.

Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-Well Kit	Storage
1	Microplate coated with secondary antibodies	8 x 12	1 month at 4°C*
2	Wash Buffer Concentrated (20X)	25ml	1 month at 4°C
3	Standard GLP-1 Peptide	2 vials	The first standard: 2-3 days at 4°C Additional dilutions: Do not store
4	Anti-GLP-1 Polyclonal Antibody	2 vials	1 month at 4°C
5	Assay Diluent B (5X)	15ml	1 month at 4°C
6	Biotinylated GLP-1 Peptide	2 vials	2-3 days at 4°C
7	HRP- Streptavidin Concentrate (250X)	600µl	Do not store and reuse
8	Positive Control	1 vial	2-3 days at 4°C
9	TMB One-Step Substrate Reagent	12ml	N/A
10	Stop Solution	8ml	N/A

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

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil
11. Plastic wrap

Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

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

1. Equilibrate 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 solution, 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

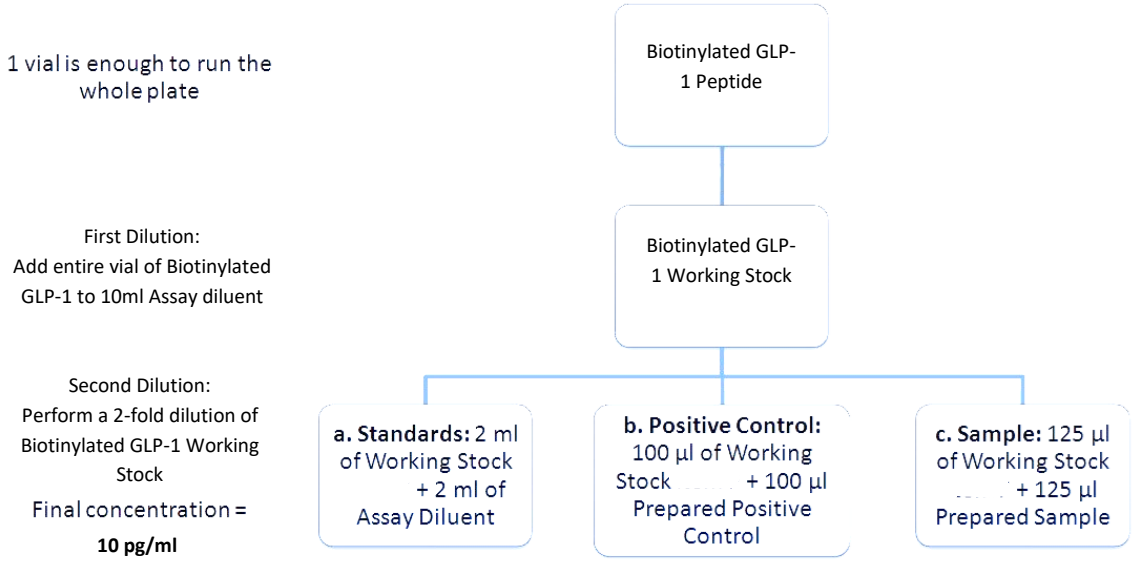
6. Briefly centrifuge the vial of Biotinylated GLP-1 before use.

7. See the image below for proper preparation of Biotinylated GLP-1 Peptide. Transfer the entire contents of the Biotinylated GLP-1 Peptide vial into a tube containing 10 ml of 1XAssay Diluent B. This is your Working Stock of Biotinylated GLP-1 Peptide. 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: Add 2 ml of Working Stock Biotinylated GLP-1 Peptide to 2 ml of 1X Assay Diluent B. The final concentration of biotinylated GLP-1 will be 10 pg/ml.

b. Second Dilution of Biotinylated GLP-1 Peptide for Positive Control: Add 100 µl of Working Stock Biotinylated GLP-1 Peptide to 100 µl of the prepared Positive Control. (See Positive Control preparation) The final concentration of biotinylated GLP-1 will be 10 pg/ml.

c. Second Dilution of Biotinylated GLP-1 Peptide for samples: Add 125 µl of Working Stock Biotinylated GLP-1 Peptide to 125 µl of prepared sample (see sample preparation). 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

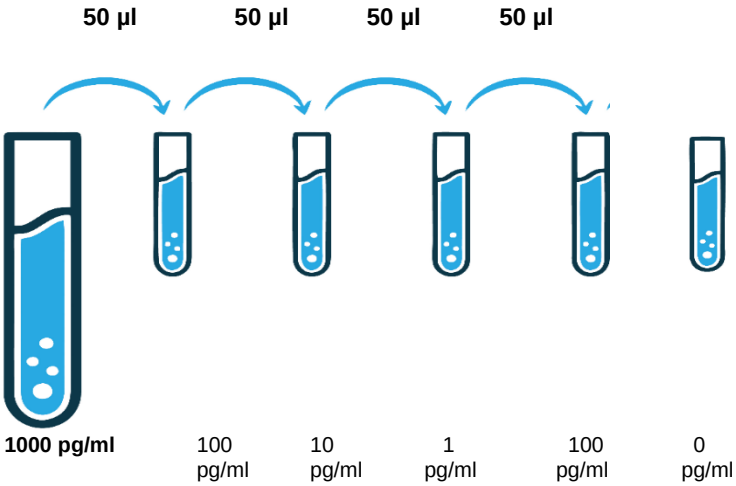
8. Label 6 microtubes with the following concentrations: 1,000 pg/ml, 100 pg/ml, 10pg/ml, 1 pg/ml, 0.1 pg/ml and 0 pg/ml. Pipette 450 µl of biotinylated GLP-1 working solution (prepared in step 7a) into each tube, except the 1,000 pg/ml (leave this one empty).
It is very important to make sure the concentration of biotinylated GLP-1 is 10 g/ml in all standards.

9. Briefly centrifuge the vial of GLP-1 Standard. Pipette 8 µl of GLP-1 Standard and 792µl of 10 pg/ml biotinylated GLP-1 working solution (prepared in step7a) into the tube labeled 1000 pg/ml. Mix thoroughly. This solution serves as the first standard (1,000 pg/ml GLP-1 standard, 10 pg/ml biotinylated GLP-1).

10. To make the 100 pg/ml standard, pipette 50 µl of the 1000 pg/ml GLP-1 standard into the tube labeled 100 pg/ml. Mix thoroughly.

11. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µl of biotinylated GLP-1 and 50 µl of the prior concentration until the 0.1 pg/ml is reached. Mix each tube thoroughly before the next transfer.

DILUTION SERIES



D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial.

13. Refer to step 7b. This is a 2-fold dilution of the Positive Control. The final concentration of biotinylated GLP-1 should still be 10 pg/ml.

The Positive Control is a mouse serum 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 Assay Genie Support. The 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

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

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 7c (125 µl of working solution biotinylated GLP-1 + 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 below for recommended dilution factors for serum: Human=2X Mouse=2X Rat=2X.

F. Preparation of Wash Buffer and HRP

15. If 20X Wash Buffer Concentrate 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 1.5 hours at room temperature with gentle shaking (1-2 cycle/sec). You may also incubate overnight at 4ºC.

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 Reagent Preparation Section C), Positive Control (see Reagent Preparation Section D) and sample (see Reagent Preparation Section E) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) overnight or at 4ºC.

5. Discard the solution and wash 4 times as directed in Step 3. 10

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 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.

3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature

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.

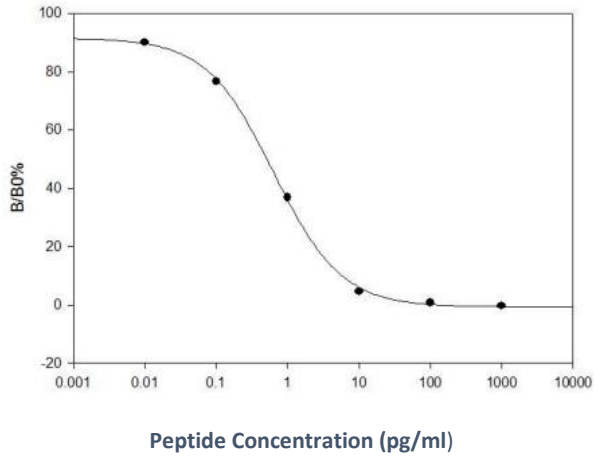
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

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)/(B0-blank OD) where
B = OD of sample or standard and
B0 = OD of zero standard (total binding)

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



B. Sensitivity

The minimum detectable concentration of GLP-1 is 0.1 pg/ml.

C. Spiking and Recovery

0.1-1000 pg/ml

D. Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <15%

E. Assay Diagram

Recommended Plate Layout

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA10	SA10	SA18	SA18	SA26	SA26	SA34	SA34
Standard1	Standard1	SA3	SA3	SA11	SA11	SA19	SA19	SA27	SA27	SA35	SA35
Standard2	Standard2	SA4	SA4	SA12	SA12	SA20	SA20	SA28	SA28	SA36	SA36
Standard3	Standard3	SA5	SA5	SA13	SA13	SA21	SA21	SA29	SA29	SA37	SA37
Standard4	Standard4	SA6	SA6	SA14	SA14	SA22	SA22	SA30	SA30	SA38	SA38
Standard5	Standard5	SA7	SA7	SA15	SA15	SA23	SA23	SA31	SA31	SA39	SA39
Pos Control	Pos Control	SA8	SA8	SA16	SA16	SA24	SA24	SA32	SA32	SA40	SA40

Key:

Blank = Buffer Only

Total Binding = Biotin-GLP-1 only

Standard 1 = 1000 pg/ml

Standard 2 = 100 pg/ml

Standard 3 = 10 pg/ml

Standard 4 = 1 pg/ml

Standard 5 = 0.1 pg/ml

Pos Control = Biotin with Positive Control

Specificity

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

Troubleshooting

Problem	Causes	Solutions
Low signal in samples	• Improper preparation of detection 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
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Standard Protein and dissolve the powder thoroughly by gently mixing
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 recommendations in the storage section of the technical manual. Keep substrate solution protected from light. • Add stop solution to each well before reading plate • Ensure correct dilution

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@ASSAYGenie.com

Web: www.ASSAYGenie.com