Catalog #81506

V.1

Crystal Chem

GLP-1 ELISA
Kit Instructions

For the quantitative determination of total GLP-1
((1-36), (7-36), and (9-36) amide)
in plasma and cell culture media

Catalog #81506
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The GLP-1 ELISA kit is for the quantitative determination of total GLP-1 ((1-36), (7-36), and (9-36) amide) in plasma and cell culture media. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

GLP-1 is a 37 amino acid peptide hormone produced from proglucagon in the small intestine and in the pancreas. The major source of GLP-1 in the body is the intestinal L cell that secretes GLP-1 as a gut hormone.

GLP-1 is a potent antihyperglycemic hormone, inducing glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion.

C. Principle of the Assay

The GLP-1 ELISA kit is a based on a sandwhich enzyme immunoassay. The 96-well plate is coated with the anti-GLP-1 antibody in which samples are added to the wells. After incubation and plate washing, labeled antibody solution is added to bind to the antibody-antigen complex. Then, HRP labeled streptoavidin (SA-HRP) is added to form antibody-antigen-biotinylated antibody complex. Finally, HRP enzyme activity is determined by TMB and the concentration of total GLP-1 is calculated.

D. Kit Storage

1. Upon receipt of the GLP-1 ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (96 wells)	1 pack
STD	Standard	1 vial
BLA	Biotin Labeled Antibody Solution	1 x 12 mL
SAHRP	SA-HRP Solution	1 x 12 mL
TMB	Enzyme Substrate Solution (TMB)	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
BUF	Buffer Solution	1 x 20 mL
WASH	Washing Solution (20X Concentrated)	1 x 50 mL
	Adhesive foil	4 sheets

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled water
Polypropylene microtubes
Volumetric flasks
Microplate shaker
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (25 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

An EDTA-2Na (1 mg/mL) additive blood collection tube is recommended for plasma collection. Plasma samples should be used as soon as possible after collection. For later testing, samples should be aliquoted and stored at below -70°C to ensure maximum stability. Avoid repeated freezing and thawing of samples.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use.
2. Standard
   The standard is provided in lyophilized form (300 fmol/vial). Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 1.24, 3.70, 11.1, 33.3, 100, and 300 pM. Once prepared, the 300 pM working standard is recommended to be stored frozen at below -70°C if not used all at once. The remaining working standards should be discarded. Working standard should be not be repeatedly thawed, so 300 pM standard should be appropriately aliquoted in appropriate volumes prior to being frozen.
3. Biotin Labeled Antibody Solution
   Provided as ready to use.
4. SA-HRP Solution
   Provided as ready to use.
5. Enzyme Substrate Solution (TMB)
   Provided as ready to use.
6. Stop Solution
   Provided as ready to use.
7. Buffer Solution
   Provided as ready to use.

8. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water.

I.2. Preparation of working standards

1. Reconsititue standard (300 fmol/vial) with 1 mL of Buffer Solution (marked "BUF") and mix thoroughly, resulting in a 300 pM working standard.
2. Dispense 0.2 mL of Buffer Solution into five polypropylene microtubes labeled 100, 33.3, 11.1, 3.70, and 1.24 pM,
3. Dispense 0.1 mL of the 300 pM standard into the 100 pM microtube, and mix thoroughly.
4. Dispense 0.1 mL of the 100 pM standard into the 33.3 pM microtube, and mix thoroughly.
5. Dispense 0.1 mL of the 33.3 pM standard into the 11.1 pM microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 0.3 mL of Buffer Solution A into one polypropylene microtube labeled 0 pM. You should now have working standards of 300, 100, 33.3, 11.1, 3.70, 1.24, and 0 pM.

Please note: Working standards should be prepared immediately prior to use. Once prepared, the 300 pM working standard is recommended to be stored frozen at below -70°C if not used all at once. The remaining working standards should be discarded. Working standard should be not be repeatedly thawed, so 300 pM standard should be appropriately aliquoted in appropriate volumes prior to being frozen.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. To maximize stability, reconstituted standard should be stored at below -70°C. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Aspirate well contents and wash three times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
2. In each well, add 100 µL of Buffer Solution.
3. In each well, add 25 µL of sample or standard and mix well by repeated pipetting.
4. Cover the wells with adhesive foil and incubate the plate for 18 hours at room temperature on a microplate shaker (shake at 100 rpm).
5. Aspirate well contents and wash three times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
6. Add 100 µL of the Biotin Labeled Antibody Solution in each well.
7. Cover the wells with adhesive foil and incubate the plate for 1 hour at room temperature on a microplate shaker (shake at 100 rpm).
8. Aspirate well contents and wash three times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
9. Add 100 µL of the SA-HRP Solution in each well.
10. Cover the wells with adhesive foil and incubate the plate for 30 mins at room temperature on a microplate shaker (shake at 100 rpm).

11. Aspirate well contents and wash three times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
12. Add 100 µL of Enzyme Substrate Solution (TMB) in each well. Cover the wells with adhesive foil and incubate the plate for 30 mins in dark room at room temperature without shaking.
13. Stop the reaction by adding 100 µL of Stop Solution to each well.
14. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the total GLP-1 concentration

1. Using computer software, construct the GLP-1 calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding GLP-1 concentration on the X axis. A higher-grade polynomial or four parametric logistic (4-PL) curve fit are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. GLP-1 concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The GLP-1 concentration is expressed in pM.
   Note: *Samples with high GLP-1 concentrations (ie. fall above the range of the assay) should be further diluted with the Buffer Solution and rerun.*

J. Performance characteristics

J.1. Assay range

The GLP-1 ELISA Kit has an assay range from 1.24 – 300 pM.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

J.3. Cross reactivity

GLP-1 Fragment	Cross Reactivity
GLP-1 (7-36) amide	100%
GLP-1 (9-36) amide	100%
GLP-1 (1-36) amide	100%

The assay has no cross reactivity with Rat GLP-2, Human GLP-2, Rat Glicentin, Human Glucagon, Rat GIP (1-42), Mouse GIP (1-42), Human GIP (1-42), Rat GIP (3-42), and Human GIP (3-42).

Warranty

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