Catalog #81512

V.1

Crystal Chem

GLP-2 ELISA
Kit Instructions

For the quantitative determination of GLP-2
in serum and plasma

Catalog #81512
96 Assays

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

Crystal Chem, Inc.
1536 Brook Drive, Suite A
Downers Grove, IL 60515, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
URL: http://www.crystalchem.com

Catalog #81512

TABLE OF CONTENTS

A. Intended Use

The GLP-2 ELISA kit is for the quantitative determination of GLP-2 in serum and plasma. 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

Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide. GLP-2 is created by cleavage of proglucagon in a process that also liberates the related glucagon-like peptide-1 (GLP-1). GLP-2 is produced by the intestinal endocrine L cell and by various neurons in the central nervous system. Intestinal GLP-2 is co-secreted along with GLP-1 upon nutrient ingestion.

This ELISA kit uses a highly specific antibody against GLP-2 and shows no cross reactivity with glucagon and GLP-1. The kit does not require sample pretreatment.

C. Principle of the Assay

The GLP-2 ELISA kit is a based on a competitive enzyme immunoassay. The 96-well plate is coated with the anti-GLP-2 antibody in which samples and labeled antigen are added to the wells for competitive immune-reaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) is added to form HRP labeled streptoavidin-biotinylated GLP-2-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by OPD and the concentration of GLP-2 is calculated.

D. Kit Storage

1. Upon receipt of the GLP-2 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 (50 ng)
ANT	Labeled antigen	1 vial
AB	GLP-2 Antibody	1 x 6 mL
SAHRP	SA-HRP Solution	1 x 12 mL
SUB	Substrate Buffer	1 x 26 mL
OPD	OPD Tablet	2 tablets
STOP	Stop Solution	1 x 12 mL
BUF	Buffer Solution	1 x 25 mL
WASH	Washing Solution (20X Concentrated)	1 x 50 mL
	Adhesive foil	3 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_{490} 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

It is recommended that serum and plasma samples 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 (50 ng/vial). Working standards should be prepared immediately prior to use as described in Section I.2. Once prepared, working standards are recommended to be stored frozen at below -70°C if not used all at once. Working standards should be not be repeatedly thawed, so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. The working standard concentrations are 0, 0.412, 1.235, 3.704, 11.11, 33.33, and 100 ng/mL.
3. Labeled Antigen
   To prepare labeled antigen solution, reconstitute labeled antigen with 6 mL of Buffer Solution (marked “BUF”).
4. GLP-2 Antibody
   Provided as ready to use.

5. SA-HRP Solution
   Provided as ready to use.
6. Substrate Buffer
   To prepare working substrate solution, dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
7. OPD Tablet
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.
9. Buffer Solution
   Provided as ready to use.
10. 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 (50 ng/vial) with 0.5 mL of Buffer Solution (marked “BUF”) and mix thoroughly, resulting in a 100 ng/mL working standard.
2. Dispense 0.2 mL of Buffer Solution into five polypropylene microtubes labeled 33.33, 11.11, 3.704, 1.235, and 0.412 ng/mL,
3. Dispense 0.1 mL of the 100 ng/mL standard into the 33.33 ng/mL microtube, and mix thoroughly.
4. Dispense 0.1 mL of the 33.33 ng/mL standard into the 11.11 ng/mL microtube, and mix thoroughly.
5. Dispense 0.1 mL of the 11.11 ng/mL standard into the 3.704 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 0.4 mL of Buffer Solution into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 100, 33.33, 11.11, 3.704, 1.235, 0.412, and 0 ng/mL.

Please note: Working standards should be prepared immediately prior to use. Once prepared, working standards are recommended to be stored frozen at below -70°C if not used all at once. Working standards should be not be repeatedly thawed, so standards 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 1 hour. Reagents should be stored at 2-8°C immediately after use. To maximize stability, reconstituted reagents 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 40 µL of labeled antigen solution.
3. In each well, add 25 µL of sample or standard and mix well by repeated pipetting.
4. In each well, add 50 µL of the GLP-2 antibody and mix well by repeated pipetting.
5. Cover the wells with adhesive foil and incubate the plate for 16-18 hours at 4°C.

6. 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.
7. Add 100 µL of the SA-HRP Solution in each well.
8. Cover the wells with adhesive foil and incubate the plate for 1 hour at room temperature. During incubation, use a microplate shaker to gently shake the plate to promote the reaction.
9. Aspirate well contents and wash five 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.
10. Add 100 µL of working substrate solution containing OPD (should be prepared immediately before use) in each well.
11. Cover the wells with adhesive foil and incubate the plate for 30 mins at room temperature.
12. Stop the reaction by adding 100 µL of Stop Solution.
13. Measure absorbance within 30 minutes using a plate reader (measure A_{490} values and subtract A_{630} values).

I.4. Determining the GLP-2 concentration

1. Using computer software, construct the GLP-2 calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding GLP-2 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-2 concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The GLP-2 concentration is expressed in ng/mL.
   Note: *Samples with high GLP-2 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-2 ELISA Kit has an assay range from 0.412 – 100 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 20%.

J.3. Cross reactivity

This ELISA kit has high specificity to GLP-2 and shows no cross reactivity with glucagon and GLP-1.

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.