Catalog #81505

V.2

Crystal Chem

Rat Glucagon ELISA Kit Instructions

For the quantitative determination of pancreatic glucagon in rat plasma

Catalog #81505
96 Assays

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

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A. Intended Use

The Rat Glucagon ELISA kit is for the quantitative determination of pancreatic glucagon in rat 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 is a 29 amino acid polypeptide hormone generated from the cleavage of proglucagon in pancreatic islet alpha cells. In intestinal L cells, proglucagon is cleaved into alternate products including glicentin, GLP-1, and GLP-2. According to many glucagon studies, it has been established that the antibody against the C-terminal fragment (19-29) of glucagon has specific binding with pancreatic glucagon, whereas the antibody against the N-terminal fragment (1-19) of glucagon has specific binding with both pancreatic and intestinal glucagon (total glucagon).

This ELISA kit uses a unique, highly specific antibody against the C-terminal fragment (19-29) to detect pancreatic glucagon and shows no cross reactivity with intestinal glucagon, GLP-1 & GLP-2. The kit does not require sample pretreatment.

C. Principle of the Assay

The Rat Glucagon ELISA kit is a based on a competitive enzyme immunoassay. The 96-well plate is coated with the anti-glucagon 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 pancreatic glucagon-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by OPD and the concentration of rat pancreatic glucagon is calculated.

D. Kit Storage

1. Upon receipt of the Rat Glucagon 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 (10 ng)
ANT	Labeled antigen	1 vial
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
BUFA	Buffer Solution A	1 x 10 mL
BUFB	Buffer Solution B	1 x 10 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_{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 (50 µL), pipetting should be done as carefully as possible. A high quality 100 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

To prepare plasma samples, whole blood should be collected into EDTA-coated tubes. Immediately add aprotinin to final concentration of 500 KIU/mL, mix well, and centrifuge at 2,000 *x g* for 20 minutes at 4°C. It is strongly recommended that 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 (10 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, 41, 123, 370, 1111, 3333, and 10000 pg/mL.

3. Labeled antigen
   To prepare labeled antigen solution, reconstitute labeled antigen with 6 mL of Buffer Solution B (marked “BUFB”).
4. SA-HRP Solution
   Provided as ready to use.
5. Substrate Buffer
   To prepare working substrate solution, dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
6. OPD Tablet
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.
8. Buffer Solution A
   Provided as ready to use.
9. Buffer Solution B
   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 (10 ng/vial) with 1 mL of Buffer Solution A (marked “BUFA”) and mix thoroughly, resulting in a 10,000 pg/mL working standard.
2. Dispense 1 mL of Buffer Solution A into five polypropylene microtubes labeled 3333, 1111, 370, 123, and 41 pg/mL,
3. Dispense 0.5 mL of the 10,000 pg/mL standard into the 3,333 pg/mL microtube, and mix thoroughly.
4. Dispense 0.5 mL of the 3,333 pg/mL standard into the 1,111 pg/mL microtube, and mix thoroughly.
5. Dispense 0.5 mL of the 1,111 pg/mL standard into the 370 pg/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 0.5 mL of Buffer Solution A into one polypropylene microtube labeled 0 pg/mL. You should now have working standards of 10000, 3333, 1111, 370, 123, 41, and 0 pg/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 30 minutes. 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. In each well, add 50 µL of sample or standard and mix well by repeated pipetting.
2. In each well, add 50 µL of labeled antigen solution and mix well by repeated pipetting.
3. Cover the wells with adhesive foil and incubate the plate for 44-48 hours at 4°C.

4. 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.
5. Add 100 µL of the SA-HRP Solution in each well.
6. 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.
7. 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.
8. Add 100 µL of working substrate solution containing OPD (should be prepared immediately before use) in each well. Cover the wells with adhesive foil and incubate the plate for 20 mins at room temperature.
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{490} values and subtract A_{630} values).

I.4. Determining the glucagon concentration

1. Using computer software, construct the glucagon calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding glucagon 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. Rat glucagon concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The glucagon concentration is expressed in pg/mL.
   Note: *Samples with high rat glucagon concentrations (ie. fall above the range of the assay) should be further diluted with the Buffer Solution A and rerun.*

J. Performance characteristics

J.1. Assay range

The Rat Glucagon ELISA Kit has an assay range from 50 – 10,000 pg/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 pancreatic glucagon and shows no cross reactivity with intestinal glucagon, GLP-1 and GLP-2.

Warranty

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