Catalog #81518

V.2

Mouse Glucagon ELISA Kit Instructions – 10µL

For the quantitative determination of glucagon
in mouse plasma, serum, and culture medium

Catalog #81518
96 Assays

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

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A. Intended Use

The Mouse Glucagon ELISA kit is for the quantitative determination of glucagon in mouse plasma, serum, and culture medium supernatant. 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 or in the brain, proglucagon is cleaved into alternate products including glicentin, GLP-1, and GLP-2. Glucagon and insulin play important, but different roles in controlling glucose levels. Increased glucagon, for example, causes a related increase in glucose levels by promoting gluconeogenesis.

This ELISA kit uses two very specific monoclonal antibodies to glucagon that have no significant (< 1%) cross-reactivity to GLP-1, GLP-2, glicentin, oxyntomodulin, or mini-glucagon. The kit does not require sample pretreatment.

C. Principle of the Assay

The Mouse Glucagon ELISA kit is based on a sandwich enzyme immunoassay. The kit uses a 96-well plate coated with a specific antibody to mouse glucagon and second, HRP-labeled, specific monoclonal antibody. HRP-labeled antibodies are added to wells that contain samples or standards. During incubation, glucagon in the sample or standard binds to the antibodies in the well and HRP-labeled antibody in solution to form a complex on the surface. After incubation and plate washing, excess HRP-labeled antibody is removed. Finally, HRP enzyme activity is determined with a TMB substrate, and the concentration of mouse glucagon is calculated.

D. Kit Storage

1. Upon receipt of the Mouse 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
HRP	HRP-labeled antibody	1 x 12 mL
SUB	Substrate solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
BUF	Buffer Solution	1 x 12 mL
WASH	Washing Solution (20X Concentrated)	1 x 50 mL
SEAL	Adhesive seal	2 sheets

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled water
Polypropylene microtubes
Volumetric flasks
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 may 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 (10 µL), pipetting should be done as carefully as possible. A high quality 20 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 standard 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-2Na coated tubes. If aprotinin is used, add it immediately to a final concentration of 500 KIU/mL, mix well, and centrifuge at 2,000 *x g* for 20 minutes at 4°C. Alternatively, commercially sold EDTA-coated collection tubes designed for GLP-1, GIP, Glucagon, and Ghrelin measurements that contain inhibitors can be used. It is strongly recommended that plasma, serum, and culture medium 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 (1 ng/mL). 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 for up to one month 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, 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 pg/mL.

3. HRP-labeled antibody
   Provided as ready to use.
4. Substrate Solution
   Provided as ready to use.
5. Stop Solution
   Provided as ready to use.
6. Buffer Solution
   Provided as ready to use.
7. 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. Reconstitute standard (1 ng/vial) with 1 mL of Buffer Solution (marked “BUF”) and mix thoroughly, resulting in a 1,000 pg/mL working standard.
2. Dispense 0.2 mL of Buffer Solution into seven polypropylene microtubes labeled 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 pg/mL
3. Dispense 0.2 mL of the 1,000 pg/mL standard into the 500 pg/mL microtube, and mix thoroughly.
4. Dispense 0.2 mL of the 500 pg/mL standard into the 250 pg/mL microtube, and mix thoroughly.
5. Dispense 0.2 mL of the 250 pg/mL standard into the 125 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 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 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 up to one month 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. Before use, mix the reagents thoroughly by gentle agitation or swirling. Remove desired number of well strips, and store remaining.

1. In each well, add 350 µL of working Wash Buffer per well and then aspirate the washing solution. Repeat this procedure two more times for a total of three washes. After the last washing, tap the plate firmly on a clean paper towel to remove any remaining liquid.
2. In the desired well, add 10 µL of sample or standard.
3. In each well, add 100 µL HRP-labeled Antibody.
4. Cover the wells with an adhesive seal and incubate the plate for 18-20 hours at 4°C without shaking.
5. Remove adhesive seal, aspirate well contents, and wash six times using 350 µL of working 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 Substrate Solution in each well.
7. Cover the wells with adhesive seal and incubate the plate for 30 minutes at room temperature in the dark without shaking.
8. Stop the reaction by adding 100 µL of Stop Solution.
9. Measure absorbance within 30 minutes using a plate reader (measure A_{450} 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 absorbance value for each standard (incl. blank) on the Y axis versus the corresponding glucagon concentration on the X axis. A four or five parametric logistic curve fit are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Mouse 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 mouse glucagon 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 Mouse Glucagon ELISA Kit has an assay range from 7.8 – 500 pg/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10% and sensitivity of < 1.1 pg/mL.

J.3. Cross reactivity

This ELISA kit has high specificity to glucagon and shows no significant (< 1%) cross reactivity with glicentin, oxyntomodulin, min-glucagon, GIP, GLP-1, GLP-2 (7-36) NH_2, or GLP-2 (9-36) NH_2.

Warranty

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