KAMIYA BIOMEDICAL COMPANY

Rat, Mouse, and Human Glucagon EIA

For the quantitative determination of Glucagon in rat, mouse and human plasma.

Cat. No. KT-381

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Rat, Mouse and Human Glucagon EIA is for the quantitative determination of Glucagon in rat, mouse and human plasma. For research use only.

INTRODUCTION
According to many studies on glucagon immunoassays, it has been established that the antibody against the C-terminal fragment (19-29) of glucagon specifically binds with pancreatic glucagon, whereas the antibody against the N-terminal fragment (1-19) of glucagon specifically binds with both pancreatic and intestinal glucagon (total glucagon). Once, 30K by Unger et al. had been widely used as an antibody specific for the C-terminal fragment of glucagon, but Nishino, Shima and Yanaihara et al. have succeeded in producing pancreatic glucagon-specific antibody using a synthetic peptide with the C-terminal fragment (19-29) of glucagon as the immunogen (in 1981). This EIA kit has been developed by using a polyclonal antibody against glucagon (19-29), a synthetic glucagon as the calibrator, and biotinylated glucagon as the labeled antigen for the measurement of rat, mouse or human glucagon in plasma. Advantages of this assay include sensitive quantification, high specificity, no interference from other components in plasma and no need for sample pre-treatment. The Glucagon Calibrator is a highly purified synthetic product (purity: > 98%) and the biotinylated peptide is purified by HPLC.

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to Glucagon and a biotin-avidin affinity system. The 96-well plate is coated with rabbit anti-Glucagon and Glucagon calibrator or samples, and biotinylated Glucagon are added to the wells for competitive immuno-reaction. After rinsing excess rat, mouse or human Glucagon, HRP-labeled streptoavidin is added to bind to the antigen-antibody complex so that HRP-labeled streptoavidin-biotinylated glucagon-antibody complexes are formed on the surface of the wells. Finally, excess HRP-labeled streptoavidins are rinsed, HRP enzyme activity is determined by OPD and the concentration of rat, mouse or human pancreatic Glucagon is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Rabbit anti-Glucagon
2. Glucagon Calibrator	Lyophilized	1 vial (10 ng/vial)	Synthetic Glucagon
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated pancreatic Glucagon
4. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
5. Substrate Buffer	Liquid	1 bottle (26 mL)	0.015% Hydrogen peroxide
6. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7. Stop Solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
8. Buffer Solution A	Liquid	1 bottle (10 mL)	Phosphate buffer with serum
9. Buffer Solution B	Liquid	1 bottle (10 mL)	Phosphate buffer
10. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
11. Plate Seal		4 sheets	

MTP[*1] ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser for ~ 0.3 mL with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Test tubes for preparation of Calibrator Solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample to avoid cross-contamination.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the Glucagon Calibrator (lyophilized rat/human/mouse Glucagon, 10 ng/ vial) with 1 mL of Buffer Solution A, giving a 10,000 pg/mL Calibrator Solution after reconstitution. 0.5 mL of the reconstituted Calibrator Solution is diluted with 1.0 mL of Buffer Solution A to yield a 3,333 pg/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 1,111, 370, 123, and 41 pg/mL. Buffer Solution A is used as the zero calibrator (0 pg/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

2. Preparation of Labeled Antigen: Reconstitute Labeled Antigen with 6 mL of Buffer Solution B.

   Note: Labeled Antigen must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Substrate Solution: Dissolve one OPD Tablet in 12 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

4. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

5. Other reagents are ready for use.

STORAGE

Store kit at 4°C. Reconstituted reagents (calibrator and labeled antigen solution) should be stored at or below -30°C if not assayed on the same day.

SPECIMEN COLLECTION AND HANDLING

Plasma samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below –30°C. Avoid repeated freeze/thaw cycles. EDTA-2Na additive blood collection tubes are recommended for the plasma collection and aprotinin 500 KIU should be added immediately for every 1 mL blood.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 100 µL of the prepared Calibrator Solutions (0, 41, 123, 370, 1,111, 3,333, 10,000 pg/mL) or samples to the wells. Then add 50 µL of Labeled Antigen to the wells. If only 50 µL sample volume is available, please see Protocol for 50 µL Sample Volume following this Assay Protocol.
3. Cover the plate with a Plate Seal and incubate at 4°C overnight (20-24 hours).
4. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.35 mL/well of Wash Solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to insure blotting free of most residual washing solution.
5. Pipette 100 µL of SA-HRP Solution into each of the wells.
6. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour. During the incubation, the plate should be rotated on a plate rotator.
7. Remove the Plate Seal, aspirate and wash the wells three times with approximately 0.35 mL/well of Wash Solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to insure blotting free of most residual washing solution.
8. Add 100 µL of Substrate Solution into the wells, cover the plate with a Plate Seal and incubate for 20 minutes at room temperature for the color reaction.
9. Add 100 µL of Stop Solution into the wells to stop the color reaction.
10. Read the optical absorbance of the wells at 490 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

During incubation except the case of 4℃ incubation and color reaction, the plate should be shaken gently by a microtiter plate shaker to promote immunoreaction.

Protocol for 50 µL Sample Volume

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 50 µL of the prepared Calibrator Solutions (0, 41, 123, 370, 1,111, 3,333, 10,000 pg/mL) or samples to the wells. Then add 50 µL of Labeled Antigen to the wells.
3. Cover the plate with a Plate Seal and incubate at 4°C for two nights (44-48 hours).

4.-10. Follow instructions 4. – 10. in the protocol above.

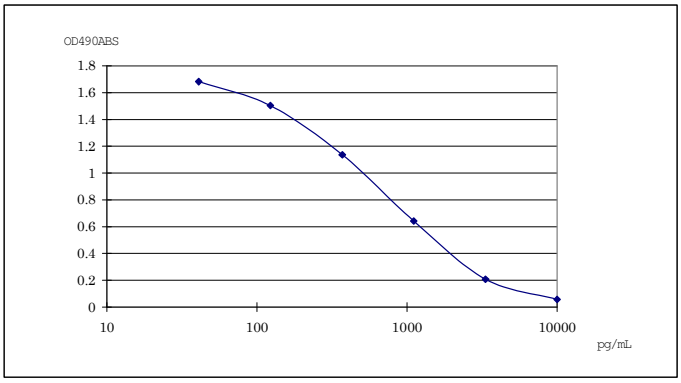
RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read Glucagon concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 10,000 pg/mL (10 ng/mL), it must be diluted with Buffer Solution A and re-assayed until the sample value is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery (Human plasma)

Glucagon Added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0.0	316	-	-
200	536	516	110
500	856	816	108
1,000	1,316	1,316	101

Precision and Reproducibility

- Intra-assay CV (%) 3.3 – 5.1
- Inter-assay CV (%) 7.3 – 18.9

Assay Range

50 – 10,000 pg/mL

Cross-Reactivity

Does not cross-react with intestinal glucagons, GLP-1 and GLP-2.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com