Glucagon (Human, Mouse, Rat) ELISA

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

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-GLUHU-E01
Size: 96 wells
Version: July 8, 2014 - September 04, 2014
1 Introduction

According to many studies on glucagon immunoassay, 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 of 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 succeeded in producing pancreatic glucagon specific antibody using synthetic peptide with the C-terminal fragment (19-29) of glucagon as immunogen in 1981. This EIA kit has been developed by using polyclonal antibody against glucagons (19-29), synthetic pancreatic glucagon as glucagon standard and biotinylated pancreatic glucagon as labeled antigen for the measurement of rat, mouse or human glucagon in plasma.

<table>
<thead>
<tr>
<th>Glucagon EIA Kit</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>▼ This assay kit can measure Glucagon within the range of 41-10000 pg/mL</td>
<td>1) Antibody coated plate</td>
</tr>
<tr>
<td>▼ The assay duration change according the sample volume: 100µL, 20-24 hr. + 1.5 hr. 50µL, 44-48 hr. + 1.5 hr</td>
<td>2) Glucagon standard</td>
</tr>
<tr>
<td>▼ With one assay kit, 41 samples can be measured in duplicate</td>
<td>3) Labeled antigen</td>
</tr>
<tr>
<td>▼ Test sample: plasma (rat, mouse, human) Sample volume: 100µL or 50 µL</td>
<td>4) SA-HRP solution</td>
</tr>
<tr>
<td>▼ The 96-well plate of this kit consisted by 8-wells strips. The kit can be used separately.</td>
<td>5) Substrate buffer</td>
</tr>
<tr>
<td>▼ Precision and reproducibility</td>
<td>6) OPD tablet</td>
</tr>
<tr>
<td>Intra-assay CV (%) 3.3-5.1</td>
<td>7) Stop solution</td>
</tr>
<tr>
<td>Inter-assay CV (%) 7.3-18.9</td>
<td>8) Buffer solution (A)</td>
</tr>
<tr>
<td>▼ Stability and storage</td>
<td>9) Buffer solution (B)</td>
</tr>
<tr>
<td>Store all of the components at 2-8°C. This kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is stated on the package.</td>
<td>10) Washing solution (concentrated)</td>
</tr>
<tr>
<td>▼ Adhesive foil</td>
<td>11)</td>
</tr>
</tbody>
</table>
II. Characteristics

This EIA kit is used for quantitative determination of rat, mouse or human pancreatic glucagon in plasma samples. The kit is characterized by sensitive quantification and high specificity. In addition, it is not influence by other components in plasma samples and sample pre-treatment is unnecessary.

Glucagon standard used in kit system is highly purified synthetic product (purity: higher than 98%) and biotinylated pancreatic glucagon is purified by HPLC.

<Specificity>
The EIA kit has high specificity to pancreatic glucagon and shows no cross reactivity with intestinal glucagon, GLP-1 and GLP-2.

<Test Principle>
This EIA kit for determination of rat, mouse or human pancreatic glucagon in plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to glucagon and biotin-avidin affinity system. The 96-well plate is coated with rabbit anti glucagon antibody. Glucagon standard or samples, and labeled antigen are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptavidin (SA-HRP) is added to form HRP labeled streptavidin-biotinylated pancreatic glucagon-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of rat, mouse or human pancreatic glucagon is calculated.
### III. Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Form</th>
<th>Quantity</th>
<th>Main Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Antibody coated plate</td>
<td>Microtiter plate</td>
<td>1 plate (96 wells)</td>
<td>Rabbit anti glucagon antibody</td>
</tr>
<tr>
<td>2. Glucagon standard</td>
<td>Lyophilized</td>
<td>1 vial (10 ng)</td>
<td>Synthetic glucagon</td>
</tr>
<tr>
<td>3. Labeled antigen</td>
<td>Lyophilized</td>
<td>1 vial</td>
<td>Biotinylated glucagon</td>
</tr>
<tr>
<td>4. SA-HRP solution</td>
<td>Liquid</td>
<td>1 bottle (12 mL)</td>
<td>HRP labeled streptoavidin</td>
</tr>
<tr>
<td>5. Substrate buffer</td>
<td>Liquid</td>
<td>1 bottle (26 mL)</td>
<td>Citrate buffer containing 0.015% hydrogen peroxide</td>
</tr>
<tr>
<td>6. OPD tablet</td>
<td>Tablet</td>
<td>2 tablets</td>
<td>ó-Phenylenediamine dihydrochloride</td>
</tr>
<tr>
<td>7. Stop solution</td>
<td>Liquid</td>
<td>1 bottle (12 mL)</td>
<td>1M H₂SO₄</td>
</tr>
<tr>
<td>8. Buffer solution (A)</td>
<td>Liquid</td>
<td>1 bottle (10 mL)</td>
<td>Phosphate buffer including serum</td>
</tr>
<tr>
<td>9. Buffer solution (B)</td>
<td>Liquid</td>
<td>1 bottle (10 mL)</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>10. Washing solution</td>
<td>Liquid</td>
<td>1 bottle (50 mL)</td>
<td>Concentrated saline</td>
</tr>
<tr>
<td>(Concentrated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Adhesive foil</td>
<td></td>
<td>4 sheets</td>
<td></td>
</tr>
</tbody>
</table>
IV. Method

<Equipment required>

1. Photometer for microtiter plate (plate reader) which can read extinction 2.5 at 490 nm

2. Microtiter plate shaker

3. Washing device for microtiter plate and dispenser with aspiration system

4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips

5. Test tubes for preparation of standard solution

6. Graduated cylinder (1,000 mL)

7. Distilled water or deionized water

<Preparatory work>

1. Preparation of plasma samples:
   EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and adds aprotinin 500KIU for every 1 mL blood immediately. It is strongly recommended that plasma samples should be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C (below -70°C is more stable.) and thawing before assay. Avoid repeated freezing and thawing of samples.

2. Preparation of standard solution:
   Reconstitute the standard (lyophilized Rat/mouse/human glucagon 10ng/vial) with 1mL of buffer solution (A), which affords 10,000 pg/mL standard solution. The reconstituted standard solution (0.5ml) is diluted with 1.0 mL of buffer solution (A), which yields 3,333 pg/mL standard solution. Repeat the same dilution to make each of standard solution 1,111, 370, 123, and 41 pg/mL. Buffer solution (A) is used as 0 pg/mL.

3. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 6 mL of buffer solution (B).

4. Preparation of substrate solution:
   Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

5. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

6. Other reagents are ready for use.
<Procedure>

<Procedure for 100μL sample volume>

1. Bring all the reagents and samples to room temperature (20-30°C) before starting assay.

2. Fill 100μL of each of standard solutions (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, then introduce 50μL of labeled antigen solution into the wells.

3. Cover the plate with adhesive foil and incubate it at 4°C for 20-24 hours. (Still, plate shaker not need)

4. After incubation, take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

5. Pipette 100μL of SA-HRP solution into the wells.

6. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker.

7. Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

8. Take off the adhesive foil, aspirate and wash the wells 3 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

9. Add 100μL of substrate solution containing OPD into the wells, cover the plate with adhesive foil and keep it for 20 minutes at room temperature for color reaction.

10. Add 100μL of stop solution into the wells to stop color reaction.

11. Read optical absorbance of the solution in the wells at 490 nm. Calculate mean absorbance values of standard solutions and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard antigen; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from the standard curve.

<Procedure for 50μL sample volume>

1. Bring all the reagents and samples return to room temperature before starting assay.

2. Fill 50μL of each of standard solutions (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, and then introduce 50μL of labeled antigen solution into the wells.

3. Cover the plate with adhesive foil and incubate it at 4°C for 44-48 hours. (Still, plate shaker not need) 4-10. Same as 4.-10. of the above mentioned procedure for 100μL sample volume.
V. Notes

1. EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and adds aprotinin 500KIU for every 1 mL blood immediately. It is strongly recommended that plasma samples should be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C (below -70°C is more stable.) and thawing before assay. Avoid repeated freezing and thawing of samples.

2. Glucagon standard solution, labeled antigen solution and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard and labeled antigen solution) should be stored at or below –30°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted.

4. Pipetting operations may affect the precision of the assay. Pipette standard solutions or samples into each well of plate precisely. Use clean test tubes or vessels in assay, and new tip must be used for each standard and sample solution to avoid cross contamination.

5. When concentration of glucagon in samples is expected to exceed 10000 pg/mL, the sample needs to be diluted with buffer solution (A) to proper concentration.

6. During incubation except the case at 4°C incubation and color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

8. Read optical absorbance of reaction solution in the wells immediately after stop color reaction.

9. For accurate quantification, plot a standard curve for each assay.

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

11. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.
VI  Performance Characteristics

Typical standard curve

Analytical recovery

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Glucagon added (pg/mL)</th>
<th>Observed (pg/mL)</th>
<th>Expected (pg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>316</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>536</td>
<td>516</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>856</td>
<td>816</td>
<td>108</td>
</tr>
<tr>
<td>4</td>
<td>1,000</td>
<td>1,316</td>
<td>1,316</td>
<td>101</td>
</tr>
</tbody>
</table>

Precision and reproducibility

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

Assay range
41-10,000 pg/mL
Ⅶ. Stability and Storage

<Storage> Store all of the components at 2-8°C.

<Shelf life> The kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is stated on the package.

-Packaging> For 96 tests per one kit.

Ⅷ. References


