Version 24-05/24

User's Manual

Human Glargine ELISA Kit

REF **DEIA-JY2133**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

In vitro quantitative determination of Glargine concentrations in serum, plasma, cell culture supernatant and other biological samples.

General Description

Insulin Glargine is a man-made insulin, which is very similar to human insulin. It has a long and steady (constant) effect on the blood sugar levels and the action lasts for an entire day.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti Glargine antibody was pre-coated onto the 96-well plate. The biotin conjugated anti Glargine antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with Glargine conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added.

After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of Glargine in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

Reagents And Materials Provided

Item	Size(48T)	Size(96T)	Storage Condition for Opened Kit
ELISA Microplate(Dismountable)	8×6	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
Lyophilized Standard	1vial	2vial	Put the lyophilized Standard and Biotin-labeled Antibody (lyophilized) into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
Biotin-labeled Antibody (Lyophilized)	1vial	1vial	
HRP-Streptavidin Conjugate(SABC, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
TMB Substrate	5ml	10ml	
Purified water	200ul	200ul	2-8°C
Sample Dilution Buffer	10ml	20ml	
Antibody Dilution Buffer	5ml	10ml	
SABC Dilution Buffer	5ml	10ml	
Stop Solution	5ml	10ml	
Wash Buffer(25X)	15ml	30ml	
Plate Sealer	3 pieces	5 pieces	
Product Description	1 copy	1 copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommenced.)
3. Automated plate washer or multi-channel pipette/5 ml pipettor (for manual washing purpose)
4. Precision single (0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date.

Specimen Collection And Preparation

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20 min at 1000 xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9 mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1 mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000 ×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3 mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15 min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH = 7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50 mM Tris + 0.9% NaCL + 0.1% SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

4. **Cell Culture Supernatant**

Collect the supernatant: Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. **Cell Lysate**

5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add precooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1 ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Lyse the cell on ice for 30 min-1 h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1 ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30 min-1 h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5 mm probe, 150-300 W, 3~5 s/time, 30s intervals for 1~2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at 80°C for future's assay.

Notes: Read notes in tissue sample.

6. **Other Biological Sample**

Centrifuge samples for 15 minutes at 1000 ×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.
2. The best sample storage condition: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25°C can decrease the effect of ice crystal (0°C) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.
3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.
4. Pretest is recommended for special samples without reference data to validate the validity.
5. Recombinant protein may not match with the capture or detection antibody in the kit, resulting in the undetectable assay.

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected)

Sample Type	Recommended Dilution Ratio	Content
Fasting serum(Healthy)	1/2-1/5	78-1225pg/ml
Postprandial serum	1/2-1/20	291-5270pg/ml

The matrix components in serum/plasma will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60 µl sample into 60 µl sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24 µl sample into 96 µl sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12 µl sample into 108 µl sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6 µl sample into 114 µl sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3 µl sample and 47 µl normal saline (0.9% NaCl) into 100 µl sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3 µl sample and 177 µl normal saline into 120 µl sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3 ul. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30 ml (15 ml for 48T) concentrated wash buffer to 750 ml (375 ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18 MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8°C.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

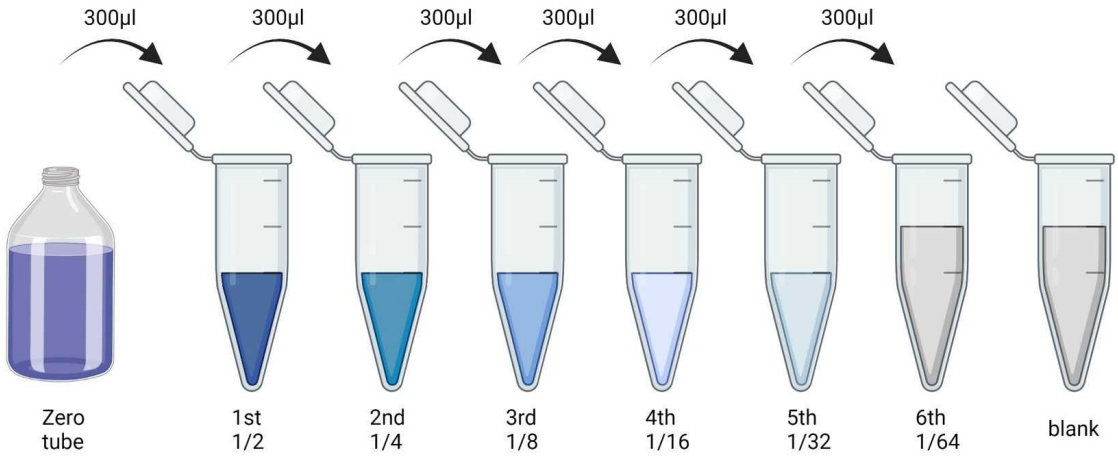
2. Standards

2.1. Centrifuge standards tube for 1min at 10000 xg. Label it as Zero tube.

2.2. Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2 min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

2.3. Centrifuge the tubes for 1min at 1000 xg, making the liquid towards the bottom of tube and removing possible bubbles.

2.4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the sample dilution buffer into each tube. Add 0.3 ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3 ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3 ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml, 7.812 pg/ml, 0 pg/ml.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. Preparation of Biotin-labeled Antibody Working Solution

The working solution should be prepared within 30 min before the assay and can't be stored for a long time.

3.1. Dissolve: Centrifuge for 1min at 2000 xg and bring down the concentrated biotin-labeled antibody to the bottom of tube. Add 130 µl purified water into tube and mix them thoroughly, after the biotin-labeled antibody is dissolved, please store it at 2-8°C.

3.2. Calculate required total volume of the working solution: 100 ul/well x quantity of wells. (It's better to prepare additional 100 ul-200 ul.)

3.3. Dilute the biotinylated detection antibody with antibody dilution buffer at 1/100 and mix them thoroughly. (e.g. Add 10µl concentrated biotin-labeled antibody into 990 µl antibody dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

The working solution should be prepared within 30 min before the assay and can't be stored for a long time.

4.1. Calculate required total volume of the working solution: 100 ul/well x quantity of wells. (It's better to prepare additional 100ul-200 ul.)

4.2. Centrifuge for 1 min at 1000 xg in low speed and bring down the concentrated SABC to the bottom of tube.

4.3. Dilute the concentrated SABC with SABC dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10 µl concentrated SABC into 990 µl SABC dilution buffer.)

Assay Procedure

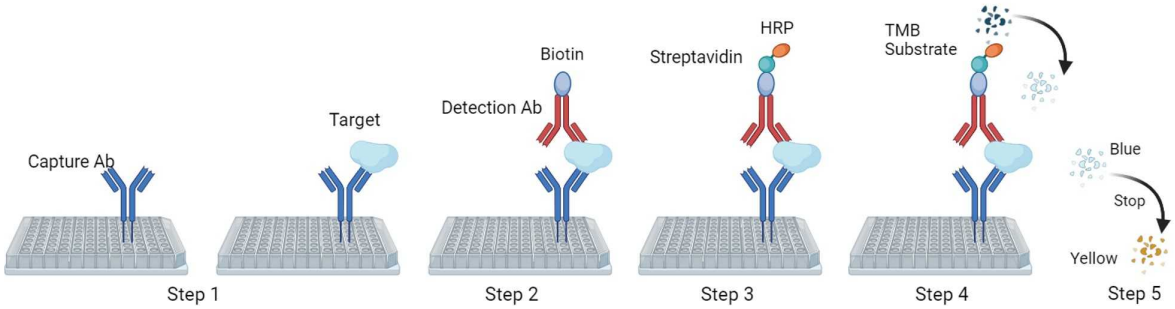
When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
2. Standards and samples loading: Aliquot 100 µl of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube into each

standard well. Also add 100 µl sample dilution buffer into the control (blank) well. Then, add 100 µl pilot samples into each sample well. Seal the plate and static incubate for 90 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)

3. Wash twice: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350 µl wash buffer into each well without immersion. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step twice.
4. Biotin-labeled Antibody: Add 100 µl biotin-labeled antibody working solution into each well. Seal the plate and static incubate for 60 minutes at 37°C.
5. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350 µl wash buffer into each well and immerse for 1 min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
6. HRP-Streptavidin Conjugate (SABC): Add 100 µl SABC working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into the 37°C incubator to equilibrate for 30 min.)
7. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 5.
8. TMB Substrate: Add 90µl TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.
9. Stop: Keep the liquid in the well after staining. Add 50 µl stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.
10. OD Measurement: Read the O.D. absorbance at 450 nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570 nm or 630 nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630 nm wavelength, the original OD450 value can be used.)

Assay Procedure Summary



Step 1: Add 100 µl standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate twice without immersion.

Step 2: Add 100 µl biotin-labeled antibody working solution into each well, seal the plate and static incubate for 60 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1 min each time.

Step 3: Add 100 µl SABC working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90 µl TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C.

(Accurate TMB visualization control is required.)

Step 5: Add 50 µl stop solution. Read at 450 nm immediately and calculate.

Detection Range

7.813-500 pg/ml

Detection Limit

4.688 pg/ml

Specificity

Specifically recognize Glargine, no obvious cross reaction with other analogues.

Precautions

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.
2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace.
3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.
4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.
6. Before confirmation, reagents from other batches or sources should not be used in this kit.
7. Don't reuse tips and tubes to avoid cross contamination.
8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.
9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.