

CODE No. 7825

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For Research Use Only. Not for use in diagnostic procedures.

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Enzyme immunoassay for the detection of insulin receptor α -subunit in human serum/plasma

Insulin receptor α -subunit ELISA TEST

CODE No. 7825

MBL

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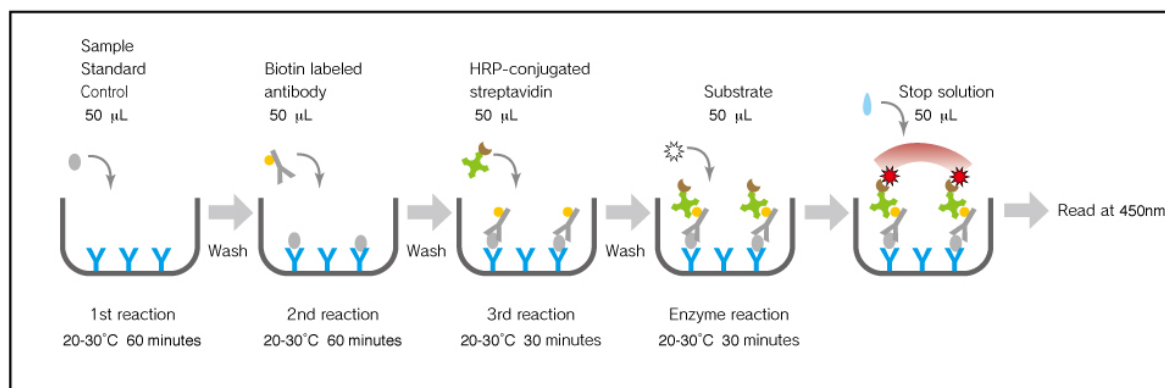
Before use, thoroughly read these Instructions.

1. Intended Use

The Insulin receptor α -subunit (IR α) ELISA TEST is an Enzyme Linked Immuno-Sorbent assay (ELISA) quantization kit for human IR α in serum and plasma.

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2. Principle



The IR α ELISA TEST measures human IR α in the serum and plasma by ELISA. Calibrators and patient serum/plasma are added to microwells coated with anti-human IR α (clone), allowing IR α to react with the immobilized antibody (Sample incubation). After washing to remove any unbound IR α and serum proteins, biotin conjugated anti IR α (clone) is added and incubated (Conjugate incubation). After washing away all unbound biotin labeled detection antibody, the peroxidase conjugated streptavidin (SA) is added and attaches to the biotin label on the bound detection antibody (Biotin-Avidin incubation). Following another washing step, the peroxidase substrate is added and incubated for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The value in each sample can be obtained by comparing the OD of the sample to the OD of the IR α Calibrator.

***3. Brief assay procedure**

<Sample incubation> Add 50 μ l of diluted sample (1:10) to each well of microwell plate (20-30 °C) 60 min.

↓
Wash

<Conjugate incubation> Add 50 μ l of Biotin conjugate solution to each well (20-30°C) 60 min.

↓
Wash

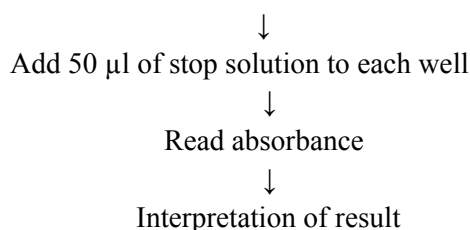
< Biotin-avidin incubation> Add 50 μ l of Peroxidase-SA solution to each well (20-30°C) 30 min.

↓
Wash

<Substrate incubation> Add 50 μ l of substrate to each well

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(20-30 °C) 30 min



4. Materials provided

Materials	Quantity (96 wells)
Microwell strips coated with anti-IR α antibody	8 wells x 12 strips
IR α Calibrator (Lyophilized) *The concentration is printed to the label.	2 vials*
Biotinylated anti-IR α antibody (101 x conc.)	0.1mL x 1 vial
POD-Conjugated Streptavidin(101 x conc.)	0.1mL x 1 vial
Assay Diluent	20 mL x 1 bottle
POD Conjugate Diluent	10 mL x 1 bottle
Wash Concentrate (10 x conc.)	100 mL x 1 bottle
Substrate Reagent (TMB/H ₂ O ₂)	10 mL x 1 bottle
Stop Solution (0.18M H ₂ SO ₄)	20 mL x 1 bottle

5. Materials and equipment required but not provided

- Microplate reader (wavelength: 450 nm, 620 nm/reference)
- Multichannel micropipette (e.g. 100 µl - 300 µl) for dispensing conjugate, substrate, and stop solution.
- Single channel / Variable volume pipette (10 µl ,100 µl & 1000 µl)
- Reagent reservoir
- Autowasher or washing bottle
- Deionized or distilled water
- One liter graduated cylinder for preparation of wash solution
- Test tubes for patient sample dilutions (e.g. 1000 µl)
- Disposable pipette tips
- Paper towels
- Basin and disinfectant
- Microplate cover

6. Analytical Precautions

- This kit is intended for Research Use Only. Not for use in diagnostic procedures. Not for human, drug, or therapeutic use.
- Do not use kit components beyond the stated expiration dates.

- Avoid contact of reagents with eyes, skin and clothing. Reagents on skin must be washed away with plenty of water. TMB contains irritant and Stop Solution consists of a 0.18 mole/L sulfuric acid, which is poisonous and corrosive.
- IR α Calibrator is derived from human serum, in which HBs antigen, HCV antibody, HIV-1 and HIV-2 antibodies have not been detected. No test method, however, can guarantee the absence of these or any other infectious agents. These reagents and all patient samples should be handled as if they are capable of transmitting AIDS, hepatitis or any other infectious diseases.
- IR α Calibrator (0.7%, 0.1mL dissolved), Biotinylated anti- IR α antibody (0.005%) and Assay Diluent (0.09%) contain sodium azide as a preservative and must be handled with caution - do not ingest or allow contact with skin or mucous membranes. Sodium azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush disposing materials containing sodium azide, with plenty of water into a drain.
- Some kit components contain animal origin materials, which are from non-infectious animals. These components, however, should be treated as potential biohazards in use and for disposal.
- All reagents must be brought to room temperature (20 -30 °C) before starting the assay.
- Do not expose the kit to direct sun during assay and storage.
- Avoid microbial and cross contamination of reagents or samples.
- Incubation temperatures above or below normal room temperature (20-30 °C), shorter or longer time periods of incubation and inaccurate dilution may give erroneous results.
- The wells must be rinsed with Wash Solution properly enough to avoid false positive.
- Carefully pipette not to foam each sample and reagent to avoid cross contamination between microwells.
- All microwell strips, which are not immediately required, should be returned to the zip lock pouch, which must be carefully resealed to avoid moisture absorption.
- Wash Concentrate may become turbid at 2-8 °C, which does not cause inconsistent results.
- Implement used for the test should be disposed or treated as shown below.
- Soak in 2% final conc. glutaraldehyde solution for more than 1 hour or soak in 0.5% Sodium hypochlorite solution (available chlorine: approx. 5000ppm) for more than 1 hour or autoclave at 121 °C for more than 20 minutes.

7. Procedure

■ PREPARATION OF REAGENTS

1. Bring all assay materials to room temperature (20-30 °C) prior to use.
2. Microwell Strips: Remove required microwell strip from pouch and place them in the frame. Promptly return unused strips to refrigerated storage.
3. Wash Solution: Prepare 1:10 dilution of The Wash Concentrate prior to use. (ex. add 100 ml of Wash Concentrate to 900 ml of distilled water). The diluted wash solution is stable for 2 weeks at 2 -8°C.
4. Biotinylated anti-IR α antibody: Dilute the Biotinylated anti-IR α antibody 1:101 by Assay Diluent. Diluted Biotinylated anti-IR α antibody can not be stored and need to dilute on each assay.
5. POD-Conjugated streptavidin: Dilute the POD-Conjugated streptavidin 1:101 by POD Conjugate Diluent. Diluted POD-Conjugated streptavidin can not be stored and need to dilute on each assay.
6. Calibrator:
 1. Dissolve Calibrator with 100 μ l of distilled water.
 - * If storage is needed, they should be aliquot and frozen below -20°C. Do not repeat freezing and thawing.
 2. Dilute 100ul of Calibrator exactory 1:10 by Assay Diluent. This diluted calibrator is used as the highest concentration calibrator.

3. Make 7 serial dilution from the highest Calibrator to make 7 calibrators (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64)
7. Do not dilute Assay Diluent, Substrate and Stop Solution, which are ready-to-use.

■ PREPARATION OF SAMPLES

1. Use fresh sera or plasma. If storage is needed, they should be aliquot and frozen below -20°C for up to one month, below -70°C for any longer storage. Do not repeat freezing and thawing.
2. Dilute each serum or plasma 1:10 by adding 20 µl of serum to 180 µl of Assay Diluent.
*Diluted samples must be used within a day.

■ ASSAY PROCEDURE

STEP 1. (SAMPLE INCUBATION)

Using the multi-channel pipette, transfer 50 µl of Assay Diluent as Blank, diluted the IRα Calibrators (7 points), and each diluted sample into the appropriate microwells.

- * Incubation starts on pipetting to the antibody-coated microwells. Pipetting should be completed as quickly as possible.

Cover wells with a plate sealer and incubate for 60 minutes at room temperature (20-30 °C).

STEP 2. (WASHING)

Aspirate or discard the well contents. Fill the well with Wash Solution and then completely aspirate or discard the contents. Wash 5 times. Tap the plate on a paper towel to remove any remaining Wash Solution. When autowasher is used, wash 5 times.

- * Each laboratory is recommended to confirm its own appropriate washing times and set-up.
- * Wash Solution should be used at 20-30 °C.

STEP 3. (CONJUGATE INCUBATION)

Pour Conjugated Reagent into a reservoir. Add 50 µl of the diluted Biotinylated anti-IRα antibody Conjugated Reagent to each well with multi-channel pipette. Cover wells with the plate sealer and incubate for 60 minutes at room temperature (20-30°C).

STEP 4. (WASHING)

Wash the microplate following the STEP 2 procedure.

STEP 5. (BIOTIN-AVIDIN INCUBATION)

Pour POD-Conjugated streptavidin into a reservoir. Add 50 µl of POD-Conjugated streptavidin to each well with multichannel pipette. Cover wells with a plate sealer and incubate at room temperature (20-25°C) for 30 minutes.

STEP 6. (WASHING)

Wash the microplate following the STEP 2 procedure.

STEP 7. (SUBSTRATE INCUBATION)

Pour Substrate into a reservoir and pipette 50 µl of the Substrate to each well with multi-channel pipette.

- *. A new disposable reservoir should be used because Substrate is easily oxidized by metal ions.

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* The Substrate, once poured in a reservoir, should not be returned to the bottle.

Cover wells with the plate sealer and incubate for 30 minutes at room temperature (20-30°C).

STEP 8. (STOP REACTION)

Pour Stop Solution into a reservoir. Pipette 50 µl of the solution to each well with multi-channel pipette.

■ **READING**

Read the absorbance of each well at 450 nm. If a dual wavelength plate reader is available, set the test wavelength at 450 nm and the reference at 620 nm.

* Reading should be done as quickly as possible after stopping the reaction.

* Ensure that the bottom of the plate is clean and dry, and that no air bubbles are present on the surface of the liquid in the wells before reading the plate.

■ **RESULTS-INTERPRETATION**

Calculate the mean absorbance value of each Calibrator and plot against log the calibrator concentration on suitable graph paper. The concentration of the samples can then be read from the calibration curve. Alternatively a suitable computer and curve-fitting program may be used.

Package size

96 wells

Storage and Stability

All kit components must be stored at 2-8°C. All reagents are stable for 12 months after manufacturing when stored at the conditions indicated.