SKU IP400150

ELISA KIT FOR MOUSE IGF1

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.0

INTENDED USE

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Insulin-like Growth Factor 1 (IGF1) in mouse serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Standard [2 vials]
Standard Diluent [20ml]
Detection Reagent A and B [120ul each]
Assay Diluent A and B [12ml each]
30X Wash solution [20ml]
TMB Substrate [9ml]
Stop Solution [6ml]
Plate Sealing Membrane [4pcs]
User manual [1pc]
Zipper bag [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C for unused kit while the others should be at 4°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Microplate reader, pipettes, microcentrifuge tubes, deionized or distilled water

SUMMARY

This kit is based on the principle of antibody sandwich enzyme linked immunosorbent assay. In brief, samples (and standards) containing the analyte are added to the micro-titre wells that are pre-coated with a protein which captures the analyte. Subsequently HRP-conjugated reagent(s) is(are) added to form an immune complex. This is followed by incubation and washing, removal of unbound enzyme, and sequential addition of chromogen which turns blue and finally changes into yellow color under the effect of acid. The color density is positively correlated with the concentration of analyte.

CHARACTERISTICS

Sample Type: Serum, Plasma, Cell Culture Supernates, Urine, Saliva Biological Fluids, etc.

Detection Range: 0.156-10ng/mL
Minimum Detection Dose: 0.061ng/ml
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%
Linearity with spiked analytes: $\rho=0.99$
Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacture
Storage: 2-8°C

Once Opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

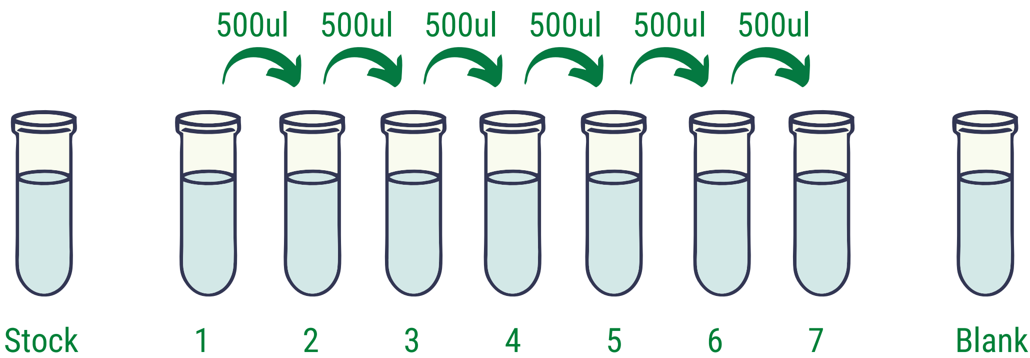
Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.
Note: Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. **Standard** - - Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 20ng/mL. Firstly dilute the stock solution to 10ng/mL (500ul Stock with 500ul standard diluent) and the diluted standard serves as the highest standard (10ng/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last EP tubes with Standard Diluent is the blank as 0ng/mL.



3. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.

4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare at least seven wells for standard, 1 well for blank. Add 100μL each of diluted standards (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.

2. Remove the liquid of each well, don't wash.

3. Add 100μL of Detection Reagent A working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37°C.

4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting or Invert the plate against absorbent paper.

5. Add 100μL of Detection Reagent B working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37°C.

6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.

7. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37oC (Don't exceed 30 minutes). Protect from light.

8. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate.

9. Remove any drop or fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid in the plate. Then, run the microplate reader and conduct measurement at 450nm immediately.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit.

As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the analyte concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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