Mouse IGF-II (for Cell Culture) ELISA kit

Cat. No.: DEIA5499
Pkg.Size: 96T

Intended use

The Mouse IGF-II ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IGF-II in cell culture supernates.

Principle Of The Test

This assay employs an antibody specific for mouse IGF-II coated on a 96-well plate. Standards and samples are pipetted into the wells and IGF-II present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IGF-II antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IGF-II bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. IGF-II Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-mouse IGF-II.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials, recombinant mouse IGF-II.
5. Detection Antibody IGF-II (Item F): 2 vials of biotinylated anti-mouse IGF-II (each vial is enough to assay half microplate).
7. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
8. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

Materials Required But Not Supplied

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 2 μl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Log-log graph paper or computer and software for ELISA data analysis.
8 Tubes to prepare standard or sample dilutions.

Storage

May be stored for up to 6 months at 2o to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at −80 °C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2o to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.
**Reagent Preparation**

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: If your samples need to be diluted, Assay diluent (Item E) should be used for dilution of cell culture supernates.
3. Assay diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C and then add 500 μl 1x Assay diluent (Item E, should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 500 ng/ml stock standard. Dissolve the powder thoroughly by a gentle mix. Add 200 μl IGF-II standard from the vial of Item C, into a tube with 466.7 μl 1x Assay Diluent to prepare a 150 ng/ml standard solution. Pipette 300 μl 1x Assay diluent into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay diluent serves as the zero standard (0 ng/ml).
5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μl of 1x Assay diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay diluent and used in step 4 of Assay Steps.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 1000-fold with 1x Assay diluent.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 15 μl of HRP-Streptavidin concentrate into a tube with 15 ml 1x Assay Diluent to prepare a 1000-fold diluted HRP-Streptavidin solution (don’t store the diluted solution for next day use). Mix well.

![Dilution Series Diagram](image)

**Assay Steps**

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 μl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room
temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 μl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 μl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 μl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

**Calculation**

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

**Typical Standard Curve**

These standard curves are for demonstration only. A standard curve must be run with each assay.
Sensitivity

The minimum detectable dose of IGF-II is typically less than 1.5 ng/ml.

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GM-CSF, IFN-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1α, IL-1β, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIK, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1y, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TIMP-1, TNF-α, TNF RI, TNF RII, TPO, VCAM-1, VEGF).

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%