Horse Transforming Growth Factor Beta 1 (TGFB1) ELISA Kit

Catalog No.: abx150474

Size: 96T

Range: 78 pg/ml - 5000 pg/ml

Sensitivity: < 28 pg/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of TGFB1 in Horse Serum, Platelet-Poor Plasma, Tissue Homogenates, Cell Lysates, Cell Culture Supernatants and other biological fluids.

Introduction: Transforming Growth Factor Beta 1 (TGFB1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation, and apoptosis. It is recommended not to use animal serum for growth of cell cultures, as it may contain high concentrations of latent TGFB1 and so the preparation of the cell culture media will affect the results. Plasma samples should be platelet-poor.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to TGFB1 is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. Biotin conjugated anti-TGFB1 antibody is used as detection antibody. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain TGFB1, biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the TGFB1 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of TGFB1 can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Stop solution: 6 ml
10. TMB substrate: 9 ml
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. Deionized or distilled water
7. 0.01 mol/L PBS (pH 7.0-7.2)
8. Tubes to prepare standard or sample dilutions
9. Absorbent filter papers
10. 100 ml and 1 liter graduated cylinders
11. 12 M HCl
12. 10 M NaOH
13. HEPES

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Platelet-poor plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C or -80°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.
- **Cell culture supernatants and other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (pH 7.0-7.2).

» If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.

» Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results. For better detection, it is highly recommended to use serum instead of plasma.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

Activation and neutralization reagent preparation

To activate latent TGFb1 to the immunoreactive form, prepare the following solutions for acid activation and neutralization:

1. Acid activation reagent: 1 M HCl (100 ml) - Slowly add 8.33 ml of 12 M HCl to 91.67 ml of deionized water. Mix well.

2. Neutralization reagent: 1.2 M NaOH/0.5 M HEPES (100 ml) - Slowly add 12 ml of 10 M NaOH to 75 ml of deionized water. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 ml with deionized water.

Sample activation procedure

Serum and Plasma:
1. To 50 µl of serum or plasma, add 10 µl of Acid activation reagent. Mix well.
2. Allow to stand at 10 minutes at room temperature.
3. Neutralize the acidified sample by adding 10 µl of Neutralization reagent. Mix well. Add 80 µl of Standard Diluent for a total volume of 150 µl. Mix well. Assay immediately.
4. The concentration read off the standard curve must be multiplied by the appropriate dilution factor, 3.

Cell culture supernatants:
1. To 100 µl of cell culture supernatants, add 20 µl of Acid activation reagent. Mix well.
2. Allow to stand at 10 minutes at room temperature.
3. Neutralize the acidified sample by adding 20 µl of Neutralization reagent for a total volume of 140 µl. Mix well. Assay immediately.
4. The concentration read off the standard curve must be multiplied by the dilution factor, 1.4.

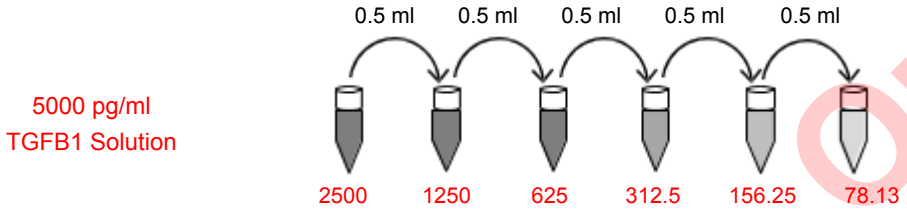
Notes:
1. Ensure that the pH of samples after neutralization is within 7.2 to 7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.
2. Activated samples must be assayed immediately. Do not freeze activated samples.
3. The solutions may be stored in polypropylene bottles at room temperature for up to one month after preparation.
4. Wear protective clothing and safety glasses during preparation or use of these reagents.
5. **Do not activate the kit standards.**

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Prepare the Standard with 1.0 ml of Standard Diluent buffer (kept for 10 min at room temperature) to make the 5000 pg/ml Standard Solution. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles. Label 6 tubes with 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.13 pg/ml. Aliquot 0.5 ml of the Standard diluent buffer into each tube. Add 0.5 ml of 5000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube, mix thoroughly, and so on.



4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with Diluent A and B respectively, and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 15 minutes prior to the experiment. Please discard after use.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles.
2. Add 100 µl of the diluted standards into the standard wells. Aliquot 100 µl Standard Diluent Buffer to the control (zero) well.
3. Add 100 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Seal the plate with a cover and incubate for 1 h at 37°C.
5. Remove the cover and discard the liquid. Do not wash.
6. Aliquot 100 µl of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.
7. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
8. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
9. Discard the solution and wash the plate 5 times with wash buffer as explained in step 7 (each time let the Wash Buffer stay for 1-2 min).

10. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
11. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, average the O.D.450 duplicate readings for each reference standard and each sample and substract the average control (zero) O.D.450 reading. The standard curve can be plotted as the relative O.D.450 of each reference standard solution (Y) vs. the respective concentration of each standard solution (X). The TGFB1 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the interpolated concentration of the sample to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid. Please ensure the tubes are completely secured and the appropriate rotor is used. Do not centrifuge if the appropriate rotor is not available.
2. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
9. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of TGFB1 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of TGFB1 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%