KAMIYA BIOMEDICAL COMPANY

Human Transforming Growth Factor Beta 1 (TGFb1) ELISA

For the quantitative determination of human TGFb1 in serum, plasma, cell culture supernates, tissue homogenates and other body fluids

Cat. No. KT-50926

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

Product Information

Human Transforming Growth Factor Beta 1 (TGFb1) ELISA

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INTENDED USE

This TGFb1 ELISA kit is intended for laboratory research use only and not for use in diagnostic or therapeutic procedures.

PRINCIPLE

TGFb1 ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with a monoclonal antibody specific for TGFb1. Calibrators or samples are then added to the microtiter plate wells and TGFb1, if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of TGFb1 present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for TGFb1 are added to each well to "sandwich" the TGFb1 immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solutions are added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain TGFb1 and enzyme-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. A calibration curve is plotted relating the intensity of the color (O.D.) to the concentration of calibrators. The TGFb1 concentration in each sample is interpolated from this calibration curve

COMPONENTS

Reagents	Quantity
Microtiter Plate	96 wells
Calibrator 1 (0 pg/mL)	1
Calibrator 2 (50 pg/mL)	1
Calibrator 3 (100 pg/mL)	1
Calibrator 4 (250 pg/mL)	1
Calibrator 5 (500 pg/mL)	1
Calibrator 6 (1,000 pg/mL)	1
Enzyme Conjugate	1 x 10 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL
Balance Solution	1 x 3 mL

Note: The balance solution is used only when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then the balance solution is a superfluous reagent.

STORAGE

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 4℃ before a centrifugation for 15 minutes at approximately 1,000 x g (3,000 rpm). Remove serum and perform the assay immediately or aliquot and store samples at -20℃ or -80℃.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g (3,000 rpm) at 4℃ within 30 minutes of collection. Store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Mince the tissues into small pieces and homogenize them in PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1,500 x g (or 5,000 rpm). Remove the supernate and assay immediately or aliquot and store samples at -20℃ or -80℃.

Cell lysates

Cells should be lysed according to the following directions.

1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2. Wash cells three times in PBS.
3. Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20℃. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
4. Centrifuge at 1,000 x g (or 3,000 rpm) for 15 minutes at 4℃ to remove cellular debris.
5. Assay immediately or store samples at -20℃ or -80℃.

Cell culture supernatants and other body fluids

Centrifuge cell culture media at 1,000 x g (or 3,000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20℃ or -80℃.

NOTE: Serum, plasma, and cell culture fluid samples to be used within 24 hours may be stored at 4°C, otherwise samples must be stored at -20°C (≤3 months) or -80°C (≤6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES.**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Microplate washer or washing bottle
3. 100 mL and 1 liter graduated cylinders.
4. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
5. 37℃ incubator.
6. Absorbent paper.
7. Distilled or de-ionized water
8. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log or semi-log, or log-logit as desired.
9. Tubes to prepare calibrator or sample dilutions.
10. Centrifuge capable of 3,000 x g.

Precautions

1. **Kamiya Biomedical Company** is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the calibration curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g. antibody targets conformational isotope rather than linear isotope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long term storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Bring all kit components and samples to room temperature (20-25°C) before use.

Samples- Please predict the concentration before assaying. If concentrations are unknown or not within the detection range a preliminary experiment is recommended to determine the optimal dilution. PBS (pH 7.0-7.2) or 0.9% physiological saline can be used as a dilution buffer.

Wash Solution

Dilute 10 mL of Wash Solution concentrate (100X) with 990 mL of de-ionized or distilled water to prepare 1,000 mL of Wash Solution (1X).

ASSAY PROCEDURE

It is recommended that all Calibrators and Samples be added in duplicate to the Microtiter Plate.

1. Secure the desired number of coated wells in the holder then add 50 µL of Calibrators or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Add 50 µL of PBS (pH 7.0-7.2) in the blank control well.
2. Dispense 5 µL of balance solution into 50 µL specimens, mix well. (**NOTE:** This step is required when the sample is cell culture fluid & body fluid & tissue homogenate; If the sample is serum or blood plasma, then this step should be skipped.)
3. Add 100 µL of Conjugate to each well (NOT blank control well). Mix well. Mixing well in this step is important. Cover and incubate for 1 hour at 37°C.
4. Wash the Microtiter Plate using one of the specified methods indicated below:
5. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of five washes. After washing, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.
6. Automated Washing: Wash plate five times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
7. Add 50 µL Substrate A and 50 µL Substrate B to each well, subsequently. Cover and incubate for 10-15 minutes at 37°C. (Avoid sunlight. If the color is not too dark please prolong the incubation time. Do not incubate longer than 30 minutes.).
8. Add 50 µL of stop solution to each well including the blank control well. Mix well.
9. Read the optical density (O.D.) at 450 nm using a microtiter plate reader immediately.

CALCULATION OF RESULTS

1. The calibration curve is used to determine the amount of an unknown sample.
2. First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of the blank control before result interpretation.
3. Construct the calibration curve by plotting the average OD for each calibrator on the horizontal or (X) axis against the concentration on the vertical (Y) axis, and draw a best fit curve using graph paper or statistical software to generate a four parameter logistic (4-PL) curve-fit or curvilinear regression of second degree. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
4. Calculate the concentration in samples corresponding to the mean absorbance from the calibration curve.
5. The sensitivity in this assay is 1.0 pg/mL.
6. This assay has high sensitivity and excellent specificity for detection of TGFb1. No significant cross-reactivity or interference between TGFb1 and analogues was observed.
   Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between TGFb1 and all the analogues, therefore, cross reaction may still exist in some cases.

Note:

1. If samples have been diluted, the concentration read from the calibration curve must be multiplied by the dilution factor.
2. If specimens generate values higher than the highest calibrator, dilute the specimens and repeat the assay.
3. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own calibration curve.

SAFETY NOTES

1. This kit contains small amount of 3,3',5,5'-Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.
2. The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.
3. Care should be taken when handling the Calibrator because of the known and unknown effects of it.
4. Care should also be taken to avoid contact of skin or eyes with other kit reagents or specimens. In the case of contact, wash immediately with water.
5. Do not pipette by mouth.
6. Avoid generation of aerosols.
7. Waste must be disposed of in accordance with federal, state and local environmental control regulations.
8. All blood components and biological materials should be handled as potentially hazardous. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.

QUALITY CONTROL

1. It is recommended that all calibrators, controls and samples be run in duplicate. Calibrators and samples must be assayed at the same time.
2. The coefficient of determination of the calibration curve should be ≥0.95.
3. Cover or cap all kit components and store at 4℃ when not in use.
4. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 4℃ to maintain plate integrity.
5. Samples should be collected in pyrogen/endotoxin-free tubes.
6. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

7. When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbance immediately after adding the stop solution.
12. Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
13. Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.

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