Human/Mouse/Rat TGF-β1 ELISA Kit

【Catalog No】EL0003Mt

【SIZE】48T/96T

【 INTENDED USE 】 For the quantitative determination of human/mouse/rat Transforming Growth Factor beta 1 (TGF-β1) concentrations in cell culture supernates, serum and plasma.

【INTRODUCTION】

Transforming growth factor beta 1 (TGF-β1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis. TGF-βs are a multifunctional set peptides that controls proliferation, differentiation, and other functions in many cell types. TGF-βs act synergistically with TGFA in inducing transformation. It also acts as a negative autocrine growth factor. Dysregulation of TGF-β activation and signaling may result in apoptosis. Many cells synthesize TGF-β and almost all of them have specific receptors for this peptide. TGF-β1, TGF-β2 and TGF-β3 all function through the same receptor signaling systems. TGF-β1 plays an important role in controlling the immune system, and shows different activities on different types of cell, or cells at different developmental stages. Most immune cells (or leukocytes) secrete TGF-β1.

TGF-β1 is related to cancer, autoimmune diseases, liver diseases, kidney diseases, diabetes, cardiovascular diseases, asthma, chronic obstructive pulmonary disorder (COPD), cystic fibrosis (CF) and so on.

【PRINCIPLE OF THE ASSAY】

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Human/Mouse/Rat TGF-β1 has been pre-coated onto a microplate. Standard, samples and biotin-linked detect antibody specific for TGF-β1 are pipetted into the wells and TGF-β1 present is bound by the immobilized antibody and detect antibody following incubation. After washing away any unbound substances, streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of TGF-β1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

【MATERIALS PROVIDED】

PART	EL0003Mt-48	EL0003Mt-96
Coated Microplate	48T	96T
Standard	1 vial	2 vials
Detect antibody	1 vial	1 vial
Standard Diluent	5ml	5ml
Streptavidin-HRP	1 vial	1 vial
Assay Buffer (10×)	10ml	10ml
TMB	6ml	11ml
Stop Solution	11ml	11ml
Washing Buffer (20×)	50ml	50ml
Adhesive Film	6	6
HCL	1 vial	1 vial
NaOH	1 vial	1 vial

Note: Components from reagent kits of different batch numbers must not be used interchangeably.

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.

2) Pipettes and pipette tips.

3) 50 μl to 300 μl adjustable multichannel micropipette with disposable tips.

4) Multichannel micropipette reservoir.

5) Beakers, flasks, cylinders necessary for preparation of reagents.

6) Deionized or distilled water.

7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8°C for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates**-Remove particulates by centrifugation at 300 × g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

Note: Animal serum used in the preparation of cell culture media may contain high levels of latent TGF-β1. For best results, do not use animal serum for growth of cell culture when assaying for TGF-β1 production. If animal serum is used as a supplement in the media, precautions should be taken to prepare the appropriate control and run the control in the immunoassay to determine the baseline concentration of TGF-β1.

2) **Serum**-Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

3) **Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Note: Neat unactivated urine samples exhibit a decrease in TGF-β1 concentration in the first 24 hours of storage (frozen or refrigerated). Care should be taken that samples are assayed under identical storage conditions and durations.

4) **Plasma**-Collect plasma using EDTA as anticoagulant. Centrifuge for 15 minutes at 1,000 × g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 × g for 10 minutes at 2 - 8℃ is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

TGF-β1 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulation levels of TGF-β1, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelet from blood. This will cause variable and irreproducible results for assays of factors contained in platelet and released by platelet activation. The recommended plasma collection protocol is designed to minimize platelet degranulation. However, since even the best methods for plasma collection may result in some platelet degranulation on occasion.

Note: Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

【SAMPLE ACTIVATION】

To activate latent TGF-β1 to immunoreactive TGF-β1, follow the activation procedure outlined below. Assay samples after neutralization (pH 7.2 - 7.6). Use polypropylene test tubes.

Note: Do not activate the kit standards. The kit standards contain active recombinant TGF-β1.

Cell culture supernates/Urine	Serum/Plasma
100 µL sample + 20 µL 1 N HCl	40 µL sample + 20 µL 1 N HCl
Mix well	Mix well
Incubate 10 minutes at RT	Incubate 10 minutes at RT
Neutralize: + 20 µL 1 N NaOH	Neutralize: + 20 µL 1 N NaOH
Mix well	Mix well
Assay immediately	Dilution: Human Serum: Active 80 µL + 720 µL Assay Buffer (1×) Mouse Serum: Active 20 µL + 480 µL Assay Buffer (1×) Human/Mouse Plasma: Active 80 µL + 80 µL Assay Buffer (1×)
The concentration read of the standard curve must be multiplied by the dilution factor, final 1.4.	The concentration read of the standard curve must be multiplied by the appropriate dilution factor. Human Serum: final 40 Mouse/Rat Serum: final 100 Plasma: final 8

Note: Activated serum and EDTA plasma samples may be stored for up to 24 hours at 2 - 8℃ before use. Activated cell culture supernate/urine samples must be assay immediately after activation.

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Streptavidin-HRP should be used within 30 minutes after dilution.

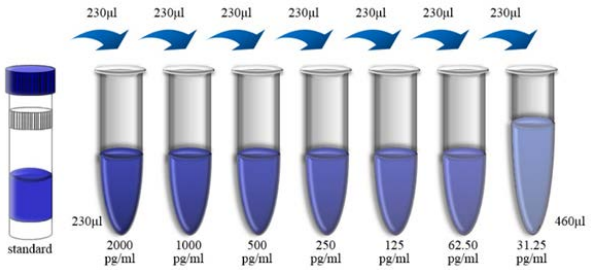
Sample Dilution: If your samples have high **TGF-β1** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

TGF-β1 Standard: Reconstitute **TGF-β1** Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 4,000 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated TGF-β1 standard (230 µl) with 230 µl of Standard Diluent creates the high standard (2,000 pg/ml). Pipette 230 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).
- **For cell culture supernates,** mixing concentrated TGF-β1 standard (230 µl) with 230 µl of cell culture medium creates the high standard (2,000 pg/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. Do not allow wells to dry.

4) Add 100 µl of 2-fold diluted Standard in duplicated. Add 100 µl of Standard Diluent/culture medium to Blank well in duplicate.

5) **Serum/Plasma:** Add 50 µl of Assay Buffer (1×) and 50 µl prediluted sample to the sample well. **Cell culture supernates:** Add 100 µl cell culture supernates to the sample well. (The dilution refers to the Sample Preparation on Page 10)

6) Add 50 µl of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) Seal the plate with an adhesive film. Incubate at room temperature (25℃±3℃) for 1.5 hours on a microplate shaker set at 300 rpm.

8) Aspirate each well and wash by filling each well with 300 µl Washing Buffer (1×), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1×) by aspirating or decanting. Invert the plate and tap it against clean paper towels.

9) Add 100 µl of diluted Streptavidin-HRP to each well.

10) Seal the plate with a fresh adhesive film. Incubate at room

temperature (25℃±3℃) for 30 minutes on a microplate shaker set at 300 rpm.

11) Repeat aspiration/wash as in step 8.

12) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature(25℃±3℃). **Protect from light.**

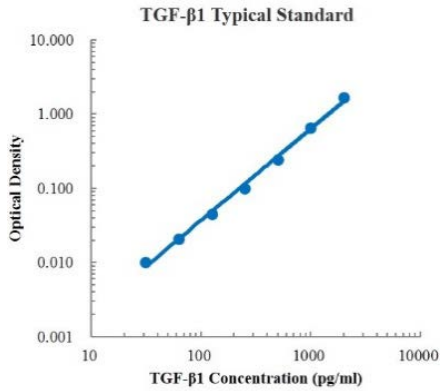
13) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

14) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

【TYPICAL DATA】

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

pg/ml	O.D.		Average	Corrected
0.00	0.025	0.024	0.025	
31.25	0.035	0.034	0.035	0.010
62.50	0.046	0.045	0.046	0.021
125.00	0.068	0.070	0.069	0.044
250.00	0.125	0.125	0.125	0.100
500.00	0.275	0.267	0.271	0.246
1000.00	0.681	0.687	0.684	0.659
2000.00	1.675	1.713	1.694	1.669



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