MULTI SCIENCES

# EasyGo!™ Human/Mouse/Rat TGF-β1 One-Step ELISA Kit

【Catalog No】EK981EGA

【SIZE】48T/96T

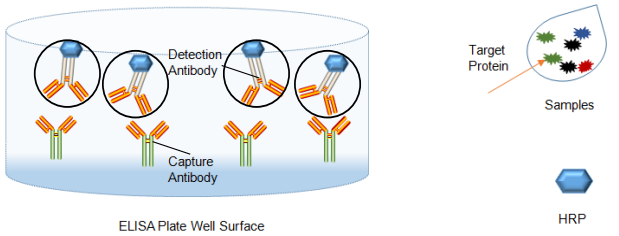
【 INTENDED USE 】 For the quantitative determination of human/mouse/rat Transforming Growth Factor beta 1 (TGF-β1) concentrations in serum and plasma.

【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 immobilized onto microwells, and two pellets of the biotin-linked detect antibody specific for TGF-β1 (light yellow) and streptavidin-HRP (purple) are pre-placed in the microwells, sealed by the adhesive film. Standard or samples are pipetted into the wells, then TGF-β1 present is bound by the immobilized antibody and detect antibody, of which the latter is conjugated with streptavidin-HRP in the incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of TGF-β1 bound by the immobilized antibody. The color development is stopped and the intensity of the color is measured by microplate reader.

【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℃ 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.

【MATERIALS PROVIDED】

| PART | PART # | EK981EGA-48 | EK981EGA-96 |
|---|---|---|---|
| Coated Microplate | EK981EGAP | 48T | 96T |
| Standard | EK981EGAS | 1 vial | 2 vials |
| Standard Diluent | E0260 | 5 mL | 5 mL |
| Assay Buffer (10×) | E0310 | 5 mL | 10 mL |
| TMB | E0230 | 6 mL | 11 mL |
| Stop Solution | E0300 | 11 mL | 11 mL |
| Washing Buffer (20×) | E0281 | 11 mL | 11 mL |
| HCl | E0302 | 1 vial | 1 vial |
| NaOH | E0303 | 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.

【SAMPLE COLLECTION AND STORAGE】

1) **Serum** – Serum separation tubes are used for serum collection, with aggregation at room temperature (25℃±3℃) for 30 minutes. Placed overnight at 2 - 8℃ to fully release TGF-β1. Centrifuge at 1000 ×g for 10 minutes. After aspirating the serum samples, detect immediately or aliquot and store at -20℃ or below.

2) **Plasma** – EDTA as an anticoagulant, within 30 minutes after blood collection, centrifuge at 1000 × g for 15 minutes to collect plasma. It is recommended to add another centrifugation at 2 - 8℃, 1000 × g for 10 minutes to completely remove platelets. Perform detection immediately, or aliquot and store at -20℃ or below.

3) Other biological samples might be suitable for use in the assay. Serum and plasma were tested with this assay.

4) 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: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃. Avoid repeated freeze-thaw cycles.

【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.**

| Serum/Plasma |
|---|
| 40 µl sample + 20 µl 1 N HCl, mix well |
| Incubate 10 minutes at RT |
| Neutralize: + 20 µl 1 N NaOH, mix well |
| Dilution:<br>Human Serum: Active 80 µl + 720 µl Assay Buffer (1×)<br>Mouse/Rat Serum: Active 20 µl + 480 µl Assay Buffer (1×)<br>Human/Mouse/Rat Plasma: Active 80 µl + 80 µl Assay Buffer (1×) |
| The concentration read of the standard curve must be multiplied by the appropriate dilution factor.<br>Human Serum: final 40<br>Mouse/Rat Serum: final 100<br>Plasma: final 8 |

**【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×)**

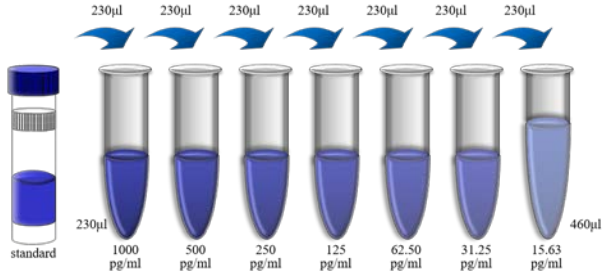
Pour the entire contents (5 ml) of the Assay Buffer (10×) into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

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

**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 =2,000 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 (1,000 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).




**【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. **In any case, avoid touching the inner surface of the microwells and gently tap the plate to ensure that the pellets on the bottom of the microwells. Do not discard the pellets.**

**3)** Adding Standard: Add 100 µL of 2-fold diluted Standard to Standard well. Add 100 µL of Standard Diluent to Blank well. **The standards/samples can be added directly from the middle opening of the plate adhesive film.**

4) Adding Samples: Add 50 µL of Assay Buffer (1×) and 50 µL sample to the sample well.

5) Incubation: Incubate at 37℃ for **1** hour, or at room temperature (25℃ ±3℃) for **2** hours, on a microplate shaker set at 300-500 rpm.

6) Washing: Removing the plate adhesive film. Aspirate each well and wash by filling each well with 300µL of Washing Buffer (1×), repeating the process 3 times for a total four washes with 60 seconds interval. 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.

7) Adding Substrate Solution: Add 100 µL of Substrate Solution to each well. Incubate for 10±5 minutes at room temperature (25±3℃). **Protect from light.**

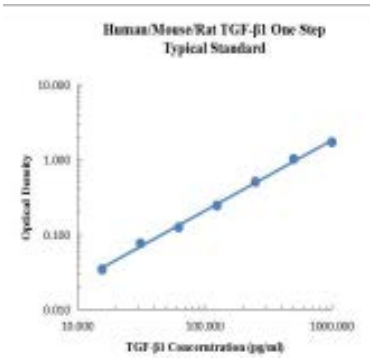
8) Stopping: Add 100 µL of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

**9)** Reading: 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 | 0.081 | 0.080 | 0.080 | |
| 15.63 | 0.116 | 0.114 | 0.115 | 0.035 |
| 31.25 | 0.158 | 0.156 | 0.157 | 0.077 |
| 62.50 | 0.203 | 0.210 | 0.207 | 0.126 |
| 125.00 | 0.315 | 0.337 | 0.326 | 0.246 |
| 250.00 | 0.569 | 0.609 | 0.589 | 0.509 |
| 500.00 | 1.069 | 1.137 | 1.103 | 1.023 |
| 1000.00 | 1.762 | 1.832 | 1.797 | 1.717 |




**MULTI SCIENCES BIOTECH CO., LTD.**

**www.multisciences.net**

**E-mail: service@multisciences.net**