EZElisa™ Rat TGF-β1/TGF beta 1 ELISA Kit

Cat #: D-AEK9006

Size: 48T / 96T

Storage: Stored at 4°C for 12 months

Product Information

Detection range: 15.63 pg/mL-1,000 pg/mL

Sensitivity: 8 pg/mL

Specificity: EZElisa™ Rat TGF-β1 ELISA Kit has high sensitivity and excellent specificity for detection of Rat TGF-β1. No significant cross-reactivity or interference between Rat TGF-β1 and analogues was observed

Applicable samples: Serum, Plasma, Cell culture supernatants

Assay Principle

Multi-functional protein that controls proliferation, differentiation and other functions in many cell types. Many cells synthesize TGF-β1 and have specific receptors for it. It positively and negatively regulates many other growth factors. It plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation. EZElisa™ Rat TGF-β1 ELISA Kit employs a double antibody sandwich method to quantitate Rat TGF-β1 in samples. An antibody specific for Rat TGF-β1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Rat TGF-β1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Rat TGF-β1 is added to the wells. After washing, proprietary Streptavidin-HRP conjugates is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, adding HRP Substrate (TMB), TMB turns blue under the catalysis of HRP, and turns yellow after adding stop solution. Measure the OD value with a microplate reader at 450nm wavelength. The TGF-β1 concentration is proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size（48T）	Size（96T）	Storage conditions
Rat TGF-β1 Microplate	48 wells	96 wells	4°C
Rat TGF-β1 Standard (lyophilized)	1	2	4°C
Sample Diluent (5×)	3.5 mL	7 mL	4°C
Assay Buffer (5×)	3.5 mL	7 mL	4°C
Rat TGF-β1 Detect Antibody (100×)	60 µL	120 µL	4°C
Streptavidin-HRP (100×)	60 µL	120 µL	4°C
HRP Substrate (TMB)	5 mL	10 mL	4°C, protected from light
Stop Solution	5 mL	10 mL	4°C
Wash Buffer (20×)	25 mL	50 mL	4°C
Plate Covers	1	2	RT

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 450 nm

·Multi channel pipette or automated microplate washer

·Incubator, refrigerated centrifuge

·Precision pipettes, disposable pipette tips

·Deionized water

Reagent Preparation

1×Sample Diluent: Sample Diluent (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the 1×Sample Diluent before use. Mix gently to avoid foaming. Store at 4°C. This solution is stable for 30 days. If your samples need to be diluted, 1×Sample Diluent is used for dilution of standard, serum and plasma samples.

1×Assay Buffer: Assay Buffer (5×) equilibrate to room temperature and dilute with deionized water 1:5 to obtain the

1×Assay Buffer before use. Mix gently to avoid foaming. Store at 4°C. This solution is stable for 30 days. 1×Assay Buffer is used for dilution of Rat TGF-β1 Detect Antibody (100×) and Streptavidin-HRP (100×).

Rat TGF-β1 Standard: Reconstitute the Rat TGF-β1 Standard in 1 mL of 1×Sample Diluent for a concentration of 1,000 pg/mL. Allow the standard to sit for a minimum of 15 min with gentle shake prior to making dilutions.

1×Rat TGF-β1 Detect Antibody: Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with 1×Assay Buffer in a clean plastic tube as needed according to the standards and samples. 1×Rat TGF-β1 Detect Antibody should be used within 30 min after dilution.

1×Streptavidin-HRP: Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with 1×Assay Buffer in a clean plastic tube as needed according to the standards and samples. 1×Streptavidin-HRP should be used within 30 min after dilution.

HRP Substrate (TMB): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Stop Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Wash Buffer: Equilibrate to room temperature and dilute with deionized water 1:20 to obtain the 1×Wash Buffer before use. Mix gently to avoid foaming. Store at room temperature. Please note that 1×Wash Buffer is stable for 30 days.

Standard curve setting: dilute 1,000 pg/mL standard with 1×Sample Diluent to 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 0 pg/mL of Rat TGF-β1 standard just as below.

NUM.	Volume of Standard	Volume of 1×Sample Diluent (μL)	The Concentration of Standard （pg/mL）
Std.1	1,000 μL of 1000 pg/mL	0	1000
Std.2	500 μL of Std.1 (1000 pg/mL)	500	500
Std.3	500 μL of Std.1 (500 pg/mL)	500	250
Std.4	500 μL of Std.1 (250 pg/mL)	500	125
Std.5	500 μL of Std.1 (125 pg/mL)	500	62.5
Std.6	500 μL of Std.1 (62.5 pg/mL)	500	31.25
Std.7	500 μL of Std.1 (31.25 pg/mL)	500	15.63
Std.8	0	500	0

Note：Always prepare a fresh set of standards per use.

Sample Preparation

1. Cell culture supernatants: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

2. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

3. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 h, they may be stored at 2 to 8°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. The strips used for testing are equilibrated to room temperature before use.

2. Acid Activation of Samples: To activate latent TGF-β1 to the immunoreactive form, the samples (but not standards) must be acidified, and then neutralized. Animal serum used in culture media may contain high levels of latent TGF-β1, so controls should be run to determine baseline concentrations of TGF-β1 in culture media.

Cell culture supernatants: Per 100 uL of sample, add 20 uL of 1 N HCl; incubate 10 min at room temperature, then neutralize with 20 uL of 1 N NaOH. When calculating final sample concentration, **correct to the dilution factor of 1.4.**

Serum or plasma: Dilute 1:5 in PBS, then treat as above for supernatants. When calculating final sample concentration, **correct to the dilution factor of 7 (1.4×5).**

3. Add 100 µL of diluted standard or sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Cover with the plate cover provided. Incubate for 2 h at room temperature.

4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well

with 1×Wash Buffer (250 µL) using a multi-channel pipette or automated microplate washer, and let it stand for 1-2 min, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

5. Add 100 µL of diluted 1×Rat TGF-β1 detect antibody to each well. Cover with the plate cover provided. Incubate for 1 h at room temperature.

6. Repeat the wash as in step 4.

7. Add 100 µL of the working dilution of 1×Streptavidin-HRP to each well. Cover the plate and incubate for 30 min at room temperature. Avoid placing the plate in direct light.

8. Repeat the wash process for five times as in step 4.

9. Add 100 µL of HRP Substrate (TMB) to each well. Cover the plate and incubate for 15 min at room temperature. Protect from light.

10. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as TMB. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1.Average the duplicate readings for each standard and sample and subtract the average zero standard (Std.8) optical density (O.D.).

2.Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Note: If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

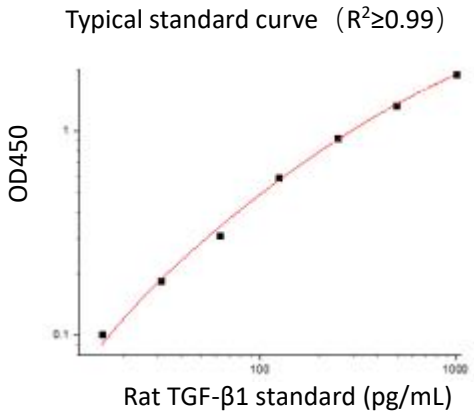

Fig.1. Standard Curve of Rat TGF-β1 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay

Precautions

1.If Sample Diluent (5×) and Assay Buffer (5×) appears to turn yellow or a small amount of precipitation, etc., it is caused by the serum contained in the reagent. Please centrifuge to remove the precipitate, which will not affect normal use.

2.Do not mix or substitute reagents with those from other lots or sources.

3.To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

4.To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.

5.Stop Solution has certain Corrosive. Please take protective measures when operating.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.