Rat TGF-β1 ELISA Kit
(rTGFβ1-ELISA)

Cat. No. EK0514

96 Tests in 8 x 12 divisible strips

Background

Transforming growth factor-β1 (TGF-β1) is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-β and essentially all of them have specific receptors for this peptide. TGF-β regulates the actions of many other peptide growth factors and can have a positive or negative regulatory affect.[1] TGF-β1 is known for its potent and diverse biological effects, including immune regulation, and cell growth and differentiation. TGF-β1 is also an important mediator of bone remodeling. TGF-β1, a potent keratinocyte growth inhibitor, has been shown to be overexpressed in keratinocytes in certain inflammatory skin diseases and has been thought to counteract the effects of other growth factors at the site of inflammation. TGF-β1, a multifunctional cytokine with fibrogenic properties, has been implicated in the pathogenesis of the vascular and target organ complications of hypertension. TGF-β1 may also regulate blood pressure via stimulation of endothelin-1 and/or renin secretion. TGF-β1 is secreted as a latent form, which consists of its mature form and a latency-associated peptide (β1-LAP) in either the presence or the absence of additional latent TGF-β1-binding protein. The standard product used in this kit is recombinant TGF-β1 with a molecular mass of 25kDa.

ScienCell's rat TGF-β1 ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat TGF-β1-specific polyclonal antibodies are pre-coated onto 8 x 12 strips. The rat-specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of rat TGF-β1 in the sample that is captured on the strips.

Size 96 Tests in 8 x 12 divisible strips

Assay type Sandwich ELISA

Range 15.6 pg/ml- 1000 pg/ml

Sensitivity < 1 pg/ml

Specificity Cross-reactivates with TGF-β2, TGF-β3, TGF-β5 <1%.

Storage Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles

Shipping Shipped on gel ice.

Expiration Four months at 4°C and eight months at -20°C.

Application For quantitative detection of rat TGF-β1 in serum, body fluids, tissue lysates or cell culture supernatants.

Activating Reagent TGF-β1 is typically in its inactive form in samples, please activate it before use. Do NOT activate recombinant TGF-β1.
Solution A: 1N HCI: add 8.33ml of 12N HCI into 91.67ml of H_2O.
Solution B: 1.2N NaOH/0.5M HEPES: add 12ml of 10N NaOH and 11.9g HEPES into 75ml of H_2O, add H_2O to adjust volume to 100ml.

Kit components
1. Lyophilized recombinant rat TGF-β1 standard: 10 ng/tube × 2.
2. One 96-well plate pre-coated with anti- rat TGF-β1 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- rat TGF-β1 antibody: 130μl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130μl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Materials Required But Not Provided
1. Microplate reader.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multi-channel pipettes are recommended for large number of samples.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
   Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
   Preparation of 0.01 M PBS: Add 8.5g NaCl, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Usage This product is for research use only. It is not approved for use in humans, animals, or *in vitro* diagnostic procedures.

Reference

1. Sporn, M. B.; Roberts, A. B.; Wakefield, L. M.; Assoian, R. K. Transforming growth factorβ: biological function and chemical structure. Science 233: 532-534, 1986.
2. Blanchette, F.; Day, R.; Dong, W.; Laprise, M.-H.; Dubois, C. M. TGFβ-1 regulates gene expression of its own converting enzyme furin. J. Clin. Invest. 99: 1974-1983, 1997.
3. Janssens, K.; Gershoni-Baruch, R.; Guanabens, N.; Migone, N.; Ralston, S.; Bonduelle, M.; Lissens, W.; Van Maldergem, L.; Vanhoenacker, F.; Verbruggen, L.; Van Hul, W. Mutations in the gene encoding the latency-associated peptide of TGFβ-1 cause Camurati-Engelmann disease. Nature Genet. 26: 273-275, 2000.

4. Li, A. G.; Wang, D.; Feng, X.-H.; Wang, X.-J. Latent TGFβ-1 overexpression in keratinocytes results in a severe psoriasis-like skin disorder. EMBO J. 23: 1770-1781, 2004.
5. Li, B.; Khanna, A.; Sharma, V.; Singh, T.; Suthanthiran, M.; August, P. TGFβ-1 DNA polymorphisms, protein levels, and blood pressure. Hypertension 33: 271-275, 1999.
6. Saito, T.; Kinoshita, A.; Yoshiura, K.; Makita, Y.; Wakui, K.; Honke, K.; Niikawa, N.; Taniguchi, N. Domain-specific mutations of a transforming growth factor (TGF)β 1 latency-associated peptide cause Camurati-Engelmann disease because of the formation of a constitutively active form of TGFβ 1. J. Biol. Chem. 276: 11469-11472, 2001.

Protocol for Rat TGF-β1 ELISA (96-well format)

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let wells dry, as this will inactivate active components in wells.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

Preparation

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernatants, tissue lysate or body fluids**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 minutes. Analyze the serum immediately or aliquot and store frozen at -70°C. **Note**: Bovine serum used in cell culture supernatants may contain TGF-β1, avoid using it if possible.

Activate the sample (if want to analyze the active form)

- **Cell culture supernatant, tissue lysate or body fluids**: add activating reagent pro rata, i.e. add 20μl of Solution A into 100μl of sample, 10 minutes later, add 20μl of Solution B. pH 7.0-7.6.
- **Serum**: add activating reagent pro rata, i.e. add 20μl of Solution A into 40μl of sample, 10 minutes later, add 20μl of Solution B. pH 7.0-7.6.
- It is unnecessary to activate the **recombinant TGF-β1**.
- **Sample is diluted after adding activating reagent, so please keep this in mind when calculating target protein concentration.**

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the

sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be mixed well with the diluent buffer**.

- **High target protein concentration (10-100 ng/ml)**. The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (1-10 ng/ml)**. The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (15.6-1000 pg/ml)**. The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤ 15.6 pg/ml)**. No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the rat TGF-β1 standard: TGF-β1 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of TGF-β1 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10,000 pg/ml of rat TGF-β1 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 1000 pg/ml of rat TGF-β1 standard solution: Add 0.1 ml of the above 10ng/ml TGF-β1 standard solution into 0.9 ml sample diluent buffer and mix thoroughly.
- 500 pg/ml→15.6 pg/ml of rat TGF-β1 standard solutions: Label 6 Eppendorf tubes with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 1000pg/ml TGF-β1 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-rat TGF-β1 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-rat TGF-β1 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard TGF-β1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of TGF-β1 amount in samples.

1. Aliquot 0.1ml per well of the 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml rat TGF-β1 standard solutions into the pre-coated 8 x 12 divisible strips. Add 0.1 ml of the sample diluent buffer into the control well (**blank well**). Add 0.1 ml of each properly diluted sample of rat serum, body fluids, tissue lysates

or cell culture supernatants to each empty well. See "**Sample Dilution Guideline**" above for details. We recommend that each rat TGF-β1 standard solution and each sample is measured in duplicate.

2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard the strips' contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-rat TGF-β1 antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method**: Discard the solution in the wells without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1 ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.
7. Wash the strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (See Step 5 for strip washing method).
8. Add 90 μl of prepared TMB color developing agent into each well and incubate the strips at 37°C in dark for 25-30 minutes (**Note**: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated rat TGF-β1 standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes to yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat TGF-β1 concentration of the samples can be interpolated from the standard curve.
Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 25-30 minutes.
5. Add TMB stop solution and read.

Typical Data Obtained from Rat TGF-β1

(TMB reaction incubate at 37°C for 25 minutes)

Concentration (pg/ml)	0.0	15.6	31.3	62.5	125	250	500	1000
Absorbance (450 nm)	0.083	0.175	0.274	0.356	0.580	1.327	1.508	1.980

Typical Rat TGF-β1 ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.