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Rat TGF-beta 2 ELISA Kit

Catalog No. EA100628
Size 96T(8×12 divisible strips)

For quantitative detection of activated rat TGF-beta 2 in cell culture supernates, serum and plasma(heparin, EDTA, citrate).

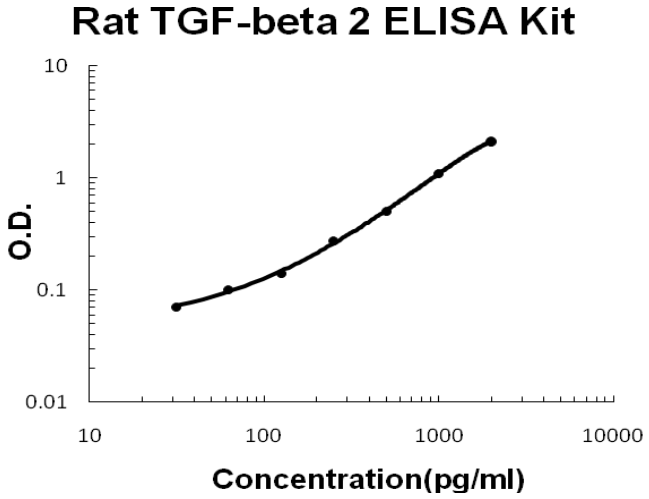
Typical Data Obtained from rat TGF-beta 2

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

Concentration(pg/ml)	0.0	31.2	62.5	125	250	500	1000	2000
O.D	0.047	0.070	0.101	0.140	0.273	0.506	1.092	2.108

Typical Rat TGF-beta 2 ELISA Kit Standard Curve

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



Range	31.2pg/ml-2000pg/ml
Sensitivity	< 10pg/ml
Specificity	Natural and recombinant rat TGF-beta 2
Cross-reactivity	No detectable cross-reactivity with other relevant proteins
Activating Reagent	TGF-beta 2 is mostly contained as inactive form in samples, please activate it before assay. Don't activate recombinant TGF-beta 2. **Solution A**: 1N HCl: add 8.33ml of 12N HCl 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.

Storage

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	304	614	1172	377	536	1223
Standard deviation	16.42	31.93	79.7	23.75	39.13	88.1
CV(%)	5.4	5.2	6.8	6.3	7.3	7.2

Principle

OriGene's rat TGF-beta 2 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TGF-beta 2 has been precoated onto 96-well plates. Standards(NSO, A303-S414) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for TGF-beta 2 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the rat TGF-beta 2 amount of sample captured in plate.

Kit Components

Description	Quantity
96-well plate precoated with anti- rat TGF-beta 2 antibody	1
Lyophilized recombinant rat TGF-beta 2 standard	10ng/tube×2
Biotinylated anti- rat TGF-beta 2 antibody	130µl(dilution 1:100)
Avidin-Biotin-Peroxidase Complex (ABC)	130µl(dilution 1:100)
Sample diluent buffer	30 ml
Antibody diluent buffer	12ml
ABC diluent buffer	12ml
TMB color developing agent	10ml
TMB stop solution	10ml

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
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 sodium chloride, 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.

Notice for Application of Kit

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.
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 will be pre-warmed in 37°C for 30 min before using.

Preparation

1. **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 supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

  Note: Animal serum used in the preparation of cell culture media may contain high levels of latent TGF-beta 2. For best results, do not use animal serum for growth of cell cultures when assaying for TGF-beta 2 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-beta 2.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma**: Collect plasma using **heparin, EDTA** or **citrate** as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

2. **Activate the sample (if want to analyze the active form)**

- **Cell culture supernates:** add activating reagent pro rata, i.e. add 20μl of Solution A into 100μl of sample, 10 min later, add 20μl of Solution B. PH 7.0-7.6.
- **Serum, plasma(heparin, EDTA** or **citrate):** add activating reagent pro rata, i.e. add 20μl of Solution A into 40μl of sample, 10 min later, add 20μl of Solution B. PH 7.0-7.6.
- It is unnecessary to activate the **recombinant TGF-beta 2.**
- **Sample was diluted partly after adding activating reagent, so please pay attention to this when calculate target protein concentration.**

3. **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 well mixed with the diluents buffer.**

- **High target protein concentration (20-200ng/ml).** The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (2-20ng/ml).** The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (31.2-2000pg/ml).** The working dilution is 1:2. i.e. Add 50µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤ 31.2pg/ml).** No dilution necessary, or the working dilution is 1:2.

4. **Reagent Preparation and Storage**

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

   a. 10,000pg/ml of rat TGF-beta 2 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

   b. 2000pg/ml of rat TGF-beta 2 standard solution: Add 0.2ml of the above 10ng/ml TGF-beta 2 standard solution into 0.8ml sample diluent buffer and mix thoroughly.

   c. 1000pg/ml→31.2pg/ml of rat TGF-beta 2 standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 2000pg/ml TGF-beta 2 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

   Note: The standard solutions are best used within 2 hours. The 10ng/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-beta 2 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

   b. Biotinylated anti-rat TGF-beta 2 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1µl Biotinylated anti-rat TGF-beta 2 antibody to 99µl antibody diluent buffer.)

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

   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

   b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µl ABC to 99µl ABC diluent buffer.)

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard TGF-beta 2 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of

TGF-beta 2 amount in samples.

1. Aliquot 0.1ml per well of the 2000pg/ml,1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml rat TGF-beta 2 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of activated rat cell culture supernates, serum or plasma(heparin, EDTA, citrate) to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each rat TGF-beta 2 standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate 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-beta 2 antibody working solution into each well and incubate the plate at 37°C for 60 min.
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 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate 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 plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min (**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-beta 2 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero 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-beta 2 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 plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 min.
5. Add TMB stop solution and read.

Background

Transforming growth factor-beta 2(TGF-beta 2) is a secreted protein known as a cytokine that performs many cellular functions and has a vital role during embryonic development. This gene is mapped to 1q41. It is an extracellular glycosylated protein. It is known to suppress the effects of interleukin dependent T-cell tumors. TGF-beta 2 is present at elevated levels in the aqueous humor of patients with primary open angle glaucoma (POAG). Studies have shown that TGF-beta 2 influences cultured trabecular meshwork cells, and it reduced outflow facility when perfused into cultured human anterior segments. In POAG, elevated expression of Gremlin by TM cells inhibited BMP4 antagonism of TGF-beta 2 and led to increased extracellular matrix deposition and elevated IOP.

Reference

1. Gottanka, J., Chan, D., Eichhorn, M., Lutjen-Drecoll, E., Ethier, C. R. Effects of TGF-beta-2 in perfused human eyes. Invest. Ophthal. Vis. Sci. 45: 153-158, 2004.
2. Wordinger, R. J., Fleenor, D. L., Hellberg, P. E., Pang, I.-H., Tovar, T. O., Zode, G. S., Fuller, J. A., Clark, A. F. Effects of TGF-beta-2, BMP-4, and gremlin in the trabecular meshwork: implications for glaucoma. Invest. Ophthal. Vis. Sci. 48: 1191-1200, 2007.