



Tgfb1 (Rat) ELISA kit

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96 assays

Version: 10

Intended for research use only

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Table of Contents

Introduction	3
Intended Use	3
Background	3
Principle of the Assay	3
General Information	4
Materials Supplied	4
Storage Instruction	4
Materials Required but Not Supplied	4
Precautions for Use	5
Assay Protocol	6
Reagent Preparation	6
Sample Preparation	6
Assay Procedure	8
Data Analysis	10
Calculation of Results	10
Performance Characteristics	10
Resources	11
References	11
Plate Layout	12

Introduction

Intended Use

For quantitative detection of activated rat TGFβ1 in cell culture supernates, serum, plasma (EDTA) and urine.

Background

Transforming growth factor-beta1 (TGF-beta1) is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. Many cells synthesize TGF-beta and essentially all of them have specific receptors for this peptide. TGF-beta regulates the actions of many other peptide growth factors and determines a positive or negative direction of their effects. TGFbeta1 is known for its potent and diverse biological effects, including immune regulation, and cell growth and differentiation. TGFbeta1 is also an important mediator of bone remodeling. TGFbeta1, 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-beta1, a multifunctional cytokine with fibrogenic properties, has been implicated in the pathogenesis of the vascular and target organ complications of hypertension. TGF-beta1 may also regulate blood pressure via stimulation of endothelin-1 and/or renin secretion. TGFbeta1 is secreted as a latent form, which consists of its mature form and a latency-associated peptide (beta1-LAP) in either the presence or the absence of additional latent TGF-beta1-binding protein. The standard product used in this kit is recombinant TGFβ1 with the molecular mass of 25 kDa.

Principle of the Assay

The ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TGFβ 1 has been precoated onto 96-well plates. Standards (CHO, A279-S390) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for TGFβ 1 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 TGFβ 1 amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
One 96-well plate precoated with anti-Rat TGFβ1 antibody	96 (8x12) wells
Lyophilized recombinant rat TGFβ1 standard	10 ng/tube x 2
Biotinylated anti- rat TGFβ1 antibody, dilution 1:100	130 μL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 μL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL
Adhesive cover	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
 - Preparation of 0.01 M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 μL of purified acetic acid or 700 μL of concentrated hydrochloric acid to 1000 mL H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
 - Preparation of 0.01 M PBS: Add 8.5 g sodium chloride, 1.4 g Na₂HPO₄ and 0.2 g NaH₂PO₄ to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
- ✓ Activating Reagent

TGFβ1 is mostly contained as inactive form in samples, please activate it before assay. Don't activate recombinant TGFβ1

Solution A: 1N HCl: add 8.33 mL of 12N HCl into 91.67 mL of H₂O.

Solution B: 1.2 N NaOH/0.5 M HEPES: add 12 mL of 10N NaOH and 11.9 g HEPES into 75 mL of H₂O,

add H₂O to adjust volume to 100 mL.

Precautions for Use

- ✓ 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.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
- ✓ Duplicate well assay is recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ Avoid using the reagents from different batches together.
- ✓ 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.

Assay Protocol

Reagent Preparation

- ✓ Reconstitution of the rat TGF β 1 standard: TGF β 1 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of TGF β 1 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
 - 10,000 pg/mL of rat TGF β 1 standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
 - 1000 pg/mL of rat TGF β 1 standard solution: Add 0.1 mL of the above 10 ng/mL TGF β 1 standard solution into 0.9 mL sample diluent buffer and mix thoroughly.
 - 500 pg/mL \rightarrow 15.6 pg/mL of rat TGF β 1 standard solutions: Label 6 Eppendorf tubes with 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 1000 pg/mL rat TGF β 1 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 may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- ✓ Preparation of biotinylated anti-rat TGF β 1 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
 - The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
 - Biotinylated anti-rat TGF β 1 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 μ L Biotinylated anti-rat TGF β 1 antibody to 99 μ L antibody diluent buffer.)
- ✓ 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: 0.1 mL/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. (i.e. Add 1 μ L ABC to 99 μ L ABC diluent buffer.)

Sample 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 supernate: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.

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 cultures 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.

- 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 min. Analyze the serum immediately or aliquot and store frozen at -70°C.
- Plasma: Collect plasma using EDTA 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.
- Urine: Aseptically collect the first urine of the day, micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

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

- Cell culture supernate, urine: add activating reagent pro rata, i.e. add 20 μL of Solution A into 100 μL of sample, 10 min later, add 20 μL of Solution B. PH 7.0-7.6.
- Serum, plasma (EDTA): add activating reagent pro rata, i.e. add 20 μL of Solution A into 40 μL of sample, 10 min later, add 20 μL of Solution B, PH 7.0-7.6.
- It is unnecessary to activate the recombinant TGFβ 1.
- Sample was diluted partly after adding activating reagent, so please pay attention to this when calculate 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 well mixed with the diluents 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.

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 TGFβ1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of TGFβ1 amount in samples.

1. Aliquot 0.1 mL per well of the 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL rat TGFβ1 standard solutions into the precoated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of rat cell culture supernatants, serum, plasma (EDTA) or urine to each empty well. See "Sample Dilution Guideline" above for details. It is recommended that each rat TGFβ1 standard solution and each sample is 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 0.1 mL of biotinylated anti-rat TGFβ1 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate 3 times with 0.01 M TBS or 0.01 M 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 0.01 M TBS or 0.01 M 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 90 μL of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15-20 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 TGFβ1 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 into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

✓ 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 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 15-20 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

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 TGFβ1 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.

Typical Data Obtained from Rat TGFβ1

Concentration (pg/mL)	0.0	15.6	31.2	62.5	125	250	500	1000
O.D.	0.033	0.133	0.234	0.384	0.709	1.187	1.869	2.299

(TMB reaction incubate at 37°C for 15 min)

Performance Characteristics

- ✓ Range
15.6 pg/mL – 1000 pg/mL
- ✓ Sensitivity
< 1 pg/mL
- ✓ Specificity
Nature and recombinant rat TGFβ1
- ✓ Cross- reactivity
Cross-reactivates with TGFβ2, TGFβ3, TGFβ5 <1%.
- ✓ 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 assay) Three samples of known concentration were tested in separate assay to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
N	16	16	16	24	24	24
Mean (pg/mL)	122	253	506	140	256	613
Standard deviation	4.15	11.4	25.81	6.72	14.08	37.39
CV (%)	3.4	4.5	5.1	4.8	5.5	6.1

Resources

References

1. Sporn, M. B.; Roberts, A. B.; Wakefield, L. M.; Assoian, R. K. Transforming growth factor-beta: biological function and chemical structure. *Science* 233: 532-534, 1986.
2. Blanchette, F.; Day, R.; Dong, W.; Laprise, M.-H.; Dubois, C. M. TGF-beta-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 TGF-beta-1 cause Camurati-Engelmann disease. *Nature Genet.* 26: 273-275, 2000.
4. Li, A. G.; Wang, D.; Feng, X.-H.; Wang, X.-J. Latent TGF-beta-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. TGF-beta-1 DNA polymorphisms, protein levels, and blood pressure. *Hypertension* 33: 271-275, 1999.

Plate Layout

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