



Product Information Sheet

Rat TGF β 1 ELISA Kit

Catalog No. EK0514

Size 96T

Range 15.6pg/ml-1000pg/ml

Sensitivity < 1pg/ml

Specificity

Cross-reacts with TGF β 2, TGF β 3, TGF β 5 <1%.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

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

Application

For quantitative detection of rat TGF β 1 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Activating Reagent

TGF β 1 is mostly contained as inactive form in samples, please activate it with acid solution if want to analyze its active form.

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

Solution B: 1.2N NaOH/0.5M HEPES: add 12ml of 10N NaOH and 11.9g HEPES into 75ml of H₂O, add H₂O to adjust volume to 100ml.

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Principle

Rat TGF β 1 ELISA kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Rat TGF β 1 specific polyclonal antibodies were precoated onto 96-well plates. The rat specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells 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 β 1 amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant rat TGF β 1 standard: 10ng/tubex2.
2. One 96-well plate precoated with anti- rat TGF β 1 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- rat TGF β 1 antibody : 130 μ l, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130 μ l, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. 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; 450 μ l of purified acetic acid or 700 μ l of concentrated hydrochloric acid to 1000ml H₂O 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 Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

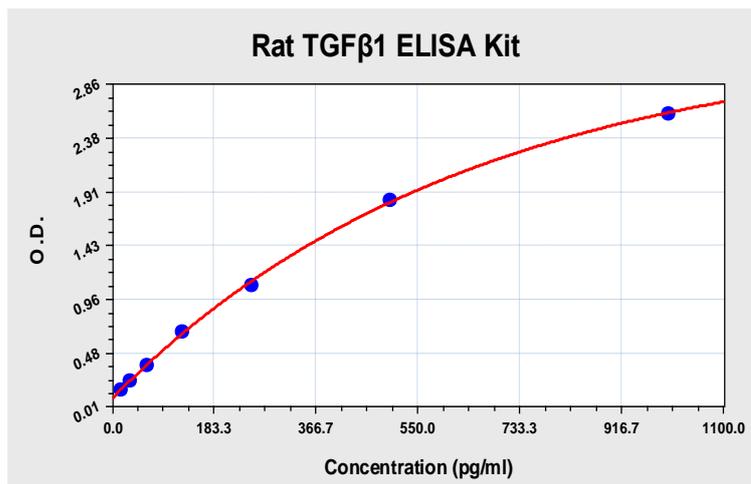
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Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. 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.

Rat TGFβ1 ELISA Kit-1X96 Well Plate Image



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.¹ TGFbeta1 is known for its potent and diverse biological effects, including immune regulation, and cell growth and differentiation.² TGFbeta1 is also an important mediator of bone remodeling.³ 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.⁴ 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.⁵ 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.⁶ The standard product used in this kit is recombinant TGFβ1 with the molecular mass of 25KDa.

Reference

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.
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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.
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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.
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)-beta 1 latency-associated peptide cause Camurati-Engelmann disease because of the formation of a constitutively active form of TGF-beta 1. *J. Biol. Chem.* 276: 11469-11472, 2001.

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