

# TGF- $\beta$ 1(Transforming Growth Factor Beta 1)

Cat# E0049– 96 well

Storage at -20°C and 4°C for 6 months

## INTENDED USE

This ELISA kit applies to the in vitro quantitative determination of TGF- $\beta$ 1 concentrations in serum, plasma and other biological fluids.

## SPECIFICITY

- Sensitivity: 0.1 ng/ml.
- Detection Range: 0.16-10 ng/ml.
- Specificity: This kit recognizes TGF- $\beta$ 1 in samples. No significant cross-reactivity or interference between TGF- $\beta$ 1 and analogues was observed.
- Repeatability: Coefficient of variation is <10%.

## INTRODUCTION

Transforming Growth Factor Beta-1 (TGF- $\beta$ 1) is a pleiotropic cytokine with potent anti-inflammatory property, and has been considered an essential risk factor in the inflammatory process of Ischemic Stroke (IS) by involving in the pathophysiological progression of lipid metabolisms, hypertension, and atherosclerosis [1]. TGF- $\beta$ 1 gene is located on chromosome 19 (q13.1–13.3), including 7 exons and 6 introns. The TGF $\beta$ -1 protein is found throughout the body but is particularly abundant in tissues that make up the skeleton, where it helps regulate the formation and growth of bone and cartilage, a tough, flexible tissue that makes up much of the skeleton during early development. TGF $\beta$ -1 is also involved in the formation of blood vessels, development of muscle tissue and body fat, wound healing, inflammatory processes in the immune system, and prevention of tumor growth [2].

1. Arpád D, Csilla V, Gabriella P, et al. The Neuroprotective Functions of Transforming Growth Factor Beta Proteins[J]. International Journal of Molecular Sciences, 2012, 13(7):8219-8258.
2. John J. Letterio and Anita B. Roberts. Regulation of immune responses by TGF- $\beta$ . Annual Review of Immunology, 1998, 16(1): 137-161.

## ALIAS

Cartilage-inducing factor,CED,Differentiation inhibiting factor,DPD1,LAP,Latency-associated peptide,Prepro transforming growth factor beta 1,TGF beta 1,TGF beta,TGF beta 1 protein,TGF-beta 1 protein,TGF-beta-1,TGF-beta-5,TGF-beta1,TGFB,Tgfb-1,tgfb1,TGFB1,TGFbeta,TGFbeta1,Transforming Growth Factor b1,Transforming Growth Factor beta 1,Transforming growth factor beta 1a,transforming growth factor beta-1,transforming growth factor,beta 1,Transforming Growth Factor- $\beta$ 1.

## **TEST PRINCIPLE**

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to TGF- $\beta$ 1. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for TGF- $\beta$ 1 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro-plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain TGF- $\beta$ 1, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of  $450 \pm 2$  nm. The OD value is proportional to the concentration of TGF- $\beta$ 1. You can calculate the concentration of TGF- $\beta$ 1 in the samples by comparing the OD of the samples to the standard curve.

## **CONTENTS & STORAGE**

An unopened kit can be stored at 4°C for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.

<b>Component</b>	<b>Specifications</b>	<b>storage</b>
Micro ELISA Plate (Dismountable)	8 well x 12 strips	
Reference Standard	2 vials	-20°C, 6 months
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 $\mu$ l	
Concentrated HRP Conjugate (100×)	1 vial, 120 $\mu$ l	-20°C (shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 ml	
Biotinylated Detection Ab Diluent	1 vial, 14 ml	4°C, 6 months
HRP Conjugate Diluent	1 vial, 14 ml	
Concentrated Wash Buffer (25×)	1 vial, 30 ml	
Activator reagent 1 (1M HCL)	1 vial, 5 ml	Room temperature, 6 months
Activator reagent 2 (1.2M NaOH/ 0.5M HEPES)	1 vial, 5 ml	
Substrate Reagent	1 vial, 10 ml	4°C (shading light)
Stop Solution	1 vial, 10 ml	4°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

**Note:** All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring.

## **OTHER SUPPLIES REQUIRED**

- Microplate reader with 450 nm wavelength filter
- High-precision transfer pipette, EP tubes and disposable pipette tips
- 37°C Incubator
- Deionized or distilled water
- Absorbent paper
- Loading slot for Wash Buffer

### **Note**

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should have a 450(±10 nm) filter installed and a detector that can detect the wavelength. The optical density should be within 0-3.5.
5. Do not mix or use components from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

## **SAMPLE COLLECTION**

- **Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2-8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.
- **Plasma:** Collect plasma using EDTA-Na<sub>2</sub> as anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!
- **Tissue homogenates:** It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight(g): PBS(ml) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5-10 min at 5000×g to get the supernatant.
- **Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000×g at 2-8°C. Collect the supernatant to carry out the assay.

## **SAMPLE ACTIVATION**

- **Serum/Plasma:** Add 40  $\mu$ l samples into 280uL Reference Standard & Sample Diluent, mix well, then add 40uL of Activator reagent 1, incubate 10 minutes at room temperature. Neutralize the acidified sample by adding 40  $\mu$ l of Activator reagent 2, mix well and test immediately.

**Note: Sample is diluted 10 times.**

- **Cell Culture Supernatant:** Add 100uL samples into 20uL Reference Standard & Sample Diluent, mix well, then add 40uL of Activator reagent 1, incubate 10 minutes at room temperature. Neutralize the acidified sample by adding 40  $\mu$ l of Activator reagent 2, mix well and test immediately.

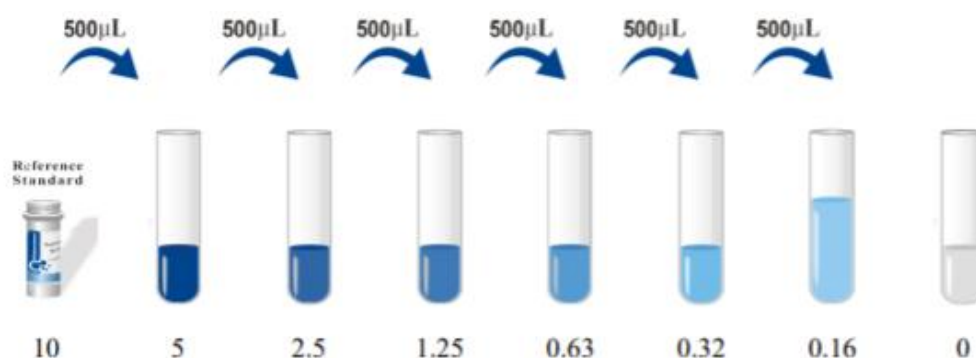
**Note: Sample is diluted 2 times.**

### **Note for sample:**

1. Samples should be assayed within 7 days when stored at 2-8 $^{\circ}$ C, otherwise samples must be divided up and stored at -20 $^{\circ}$ C ( $\leq$ 1 month) or -80 $^{\circ}$ C ( $\leq$ 3 months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.
5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.
6. Activated samples should be stored at 4 $^{\circ}$ C and tested within 2h.

## REAGENT PREPARATION

1. Bring all reagents to room temperature (18-25°C) before use. Preheat microplate reader for 15 min before OD measurement.
2. **Wash Buffer:** Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1min. Add 1.0ml of Reference Standard & Sample Diluent, let it stand for 10min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 10 ng/ml. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10、5、2.5、1.25、0.63、0.32、0.16、0 pg/ml. Dilution method: Take 7 EP tubes, add 500 μl of Reference Standard & Sample Diluent to each tube. Pipette 500 μl of the 10 ng/ml working solution to the first tube and mix up to produce a 5 ng/ml working solution. Pipette 500 μl of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 μl/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100 μl/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100×Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

## **ASSAY PROCEDURE (A brief assay procedure is on the 10<sup>th</sup> page)**

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µl for each well). Add the samples to the other wells (100 µl for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. **Note:** solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible
2. Remove the liquid out of each well, do not wash. Immediately add 100 µl of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.
3. Aspirate or decant the solution from each well › add 350 µl of **wash buffer** to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. **Note:** a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. **Note:** the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
7. Add 50 µl of **Stop Solution** to each well. **Note:** Adding the stop solution should be done in the same order as the substrate solution..
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

## **CALCULATION OF RESULTS**

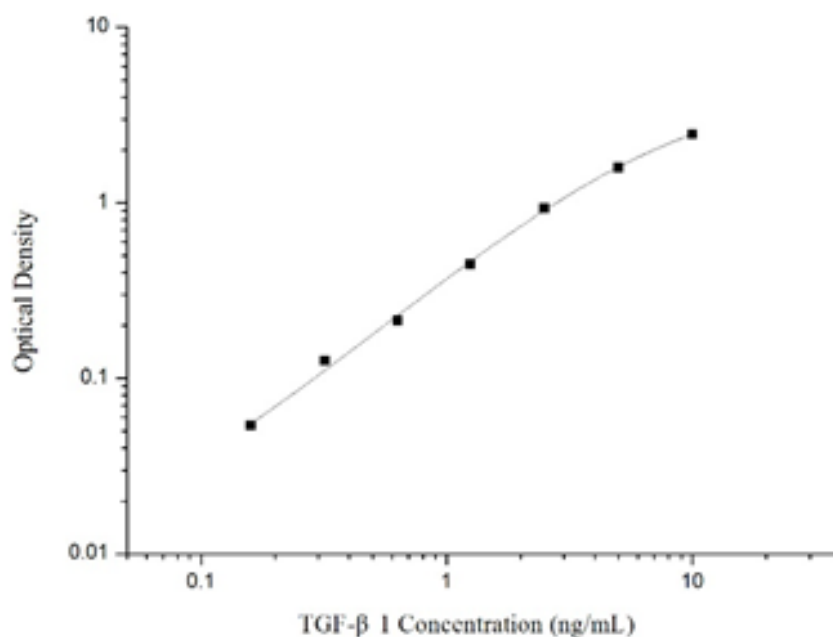
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

## **TYPICAL DATA**

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(ng/ml)	10	5	2.5	1.25	0.63	0.32	0.16	0
OD	2.515	1.645	0.988	0.506	0.271	0.183	0.111	0.057
Corrected OD	2.458	1.588	0.931	0.449	0.214	0.126	0.054	-



## **SAMPLE VALUE**

**Serum/Plasma** –Samples from apparently healthy human, mouse, rat, porcine, rabbit, canine were evaluated for detectable levels of TGF-β1 in this assay.

Sample Type	Source	Range	Dilution Factor
Serum	Healthy Human	22.8-46.1 ng/ml	10
Plasma	Healthy Human	12.7-43.1 ng/ml	10
Serum	Healthy Mouse	66.1-82.9 ng/ml	10
Serum	Healthy Rat	35.4-66.5 ng/ml	10
Serum	Healthy Porcine	4.9-10.2 ng/ml	10
Serum	Healthy Rabbit	2.9-6.5 ng/ml	10
Serum	Healthy Canine	34.5-84.2 ng/ml	10
Liver	Healthy Mouse	2.4-10.1 ng/ml	5
Liver	Healthy Rat	3.9-22.4 ng/ml	5

## **PRECISION**

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level TGF- $\beta$ 1 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level TGF- $\beta$ 1 were tested on 3 different plates, 20 replicates in each plate.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	0.41	1.07	4.97	0.47	1.25	4.24
SD	0.02	0.05	0.22	0.03	0.06	0.2
CV (%)	4.88	4.67	4.43	6.38	4.8	4.72

## **RECOVERY**

The recovery of TGF- $\beta$ 1 spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	94-110	102
EDTA plasma (n=5)	90-102	96
Cell culture media (n=5)	95-106	98

## **LINEARITY**

Samples were spiked with high concentrations of TGF- $\beta$ 1 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum(n=5)	Plasma(EDTA)(n=5)	Cell culture media (n=5)
		1:2	Range (%)	99-108
	Average (%)	105	102	97
1:4	Range (%)	95-109	90-100	97-105
	Average (%)	102	96	100
1:8	Range (%)	89-103	89-104	96-108
	Average (%)	97	97	102
1:16	Range (%)	88-102	98-108	92-105
	Average (%)	94	104	100



## **TROUBLESHOOTING**

<b>Problem</b>	<b>Causes</b>	<b>Solutions</b>
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.	
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

## **SUMMARY**

1. Add 100 µl standard or sample to each well. Incubate for 90 min at 37°C.
2. Remove the liquid. Add 100 µl Biotinylated Detection Ab. Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times.
4. Add 100 µl HRP Conjugate. Incubate for 30 min at 37°C.
5. Aspirate and wash 5 times.
6. Add 90 µl Substrate Reagent. Incubate for 15 min at 37°C.
7. Add 50 µl Stop Solution. Read at 450 nm immediately.
8. Calculation of results

## **DECLARATION**

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.