

## Mouse TGF-beta1 Sandwich ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** KE10005

**Size:** 96T

**Sensitivity:** 16.0 pg/mL

**Range:** 31.25-2000 pg/mL

**Usage:** For the quantitative detection of mouse TGF-beta1 concentrations in serum, plasma and cell culture supernatant.

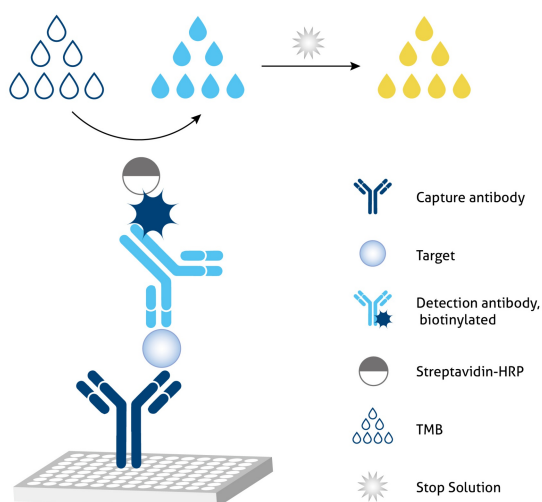
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## 1. Background

TGF-beta is a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. TGF-beta is produced by a number of cell types including regulatory T cells, fibroblasts, epithelial cells, and endothelial cells. TGF-beta acts synergistically with TGFA in inducing transformation. It also acts as a negative autocrine growth factor. TGF-beta plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts. Mice lacking TGF-beta develop severe multifocal inflammatory disease, yolk sac defects and colon cancer.

## 2. Principle



### Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

## 4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months. <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days. <b>Please use a new standard for each assay.</b>
Protein standard - 4000 pg/bottle; lyophilized	2 bottles	
Detection antibody, biotinylated (100×) - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

\* Centrifugation immediately before use

## 5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

## 6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

## 7. Reagent Preparation

- 7.1 **Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.
- 7.2 **Detection Antibody (1X):** Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).
- 7.3 **Streptavidin-HRP (1X):** Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100

dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

**7.4 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

To activate latent TGF-beta1 to the immunoreactive form, prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month.

1 N HCL (100 mL) - To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCL. Mix well.

1.2 N NaOH/ 1 0.5 M HEPES (100 mL) - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water.

For each new lot of acidification and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.

Cell culture supernatant	Serum/Plasma
To 100 µL of cell culture supernate, add 20 µL of 1 N HCL.	To 50 µL serum/plasma, add 25 µL of 1 N HCL.
Mix well.	Mix well.
Incubate 10 minutes at room temperature .	Incubate 10 minutes at room temperature .
Neutralize the acidified sample by adding 20 µL of 1.2 N NaOH/0.5M HEPES.	Neutralize the acidified sample by adding 25 µL of 1.2 N NaOH/0.5M HEPES.
Mix well Mix well.	Mix well Mix well.
Assay immediately.	Prior to the assay, dilute the activated sample with Sample Diluent PT 1-ef. See the following data for suggested dilutions.
The dilution factor is 1.4.	The dilution factors is 2.

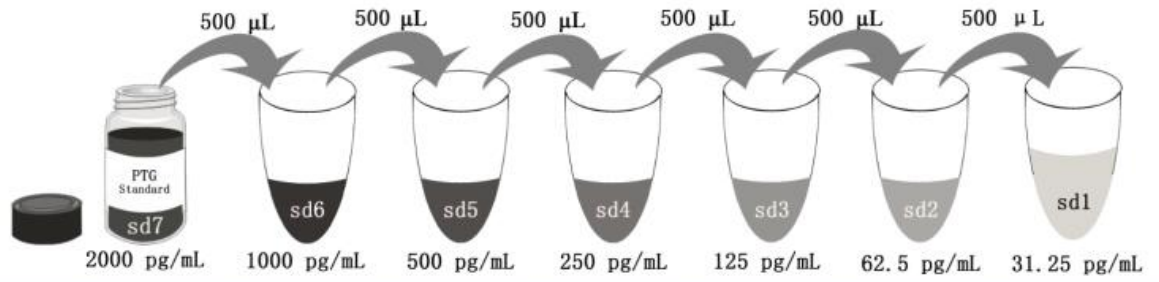
Recommended Dilution for different sample types: 1:4 or 1:8 is recommended for activated cell culture supernatant; 1:120 or 1:240 is recommended for activated mouse serum and plasma.

**Cell culture supernatant samples** (human and non-human) require a 4-fold or 8-fold dilution in Sample Diluent PT 1-ef. A suggested 4-fold dilution is 150 µL of activated sample (diluted 1:1.4) + 450 µL of Sample Diluent PT 1-ef (final dilution factor of sample is 5.6) or 8-fold dilution is 75 µL of activated sample (diluted 1:1.4) + 525 µL of Sample Diluent PT 1-ef (final dilution factor of sample is 11.2).

**Mouse serum and plasma samples** require a 120-fold or 240-fold dilution in Sample Diluent PT 1-ef. A suggested 120-fold dilution is 5 µL of activated sample (diluted 1:2) + 595 µL of Sample Diluent PT 1-ef (final dilution factor of sample is 240) or 240-fold dilution is 2.5 µL of activated sample (diluted 1:2) + 597.5 µL of Sample Diluent PT 1-ef (final dilution factor of sample is 480).

**7.5 Standard Serial Dilution:**

Add 2 mL Sample Diluent PT1-ef in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	<b>2000 µL</b>	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## 8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, it is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

### 8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

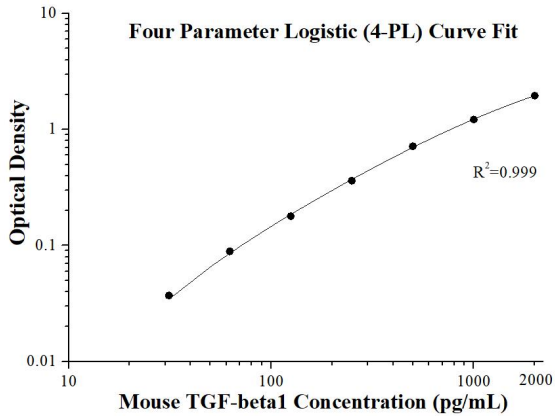
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

## 9. Validation Data

### 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.046 0.045	0.046	-
31.25	0.085 0.08	0.083	0.037
62.5	0.136 0.133	0.135	0.09
125	0.227 0.222	0.225	0.18
250	0.402 0.413	0.408	0.363
500	0.765 0.759	0.762	0.717
1000	1.314 1.215	1.265	1.219
2000	2.07 1.94	2.005	1.960

### 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	72.4	3.3	4.5
2	20	154.8	11.1	7.2
3	20	295.8	19.7	6.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	58.9	4.4	7.5
2	24	128.0	6.8	5.3
3	24	248.0	17.4	7.0

## 9.3 Recovery

The recovery of mouse TGF-beta1 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:300	94	88-109
	1:600	101	84-115
Cell culture supernatant	1:4	93	80-102
	1:8	89	84-98

## 9.4 Sample values

**Mouse serum** - Mouse serum samples were evaluated for the presence of mouse TGF-beta1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Mouse serum (n=10)	66.8	41.2-99.3

### Cell culture supernatant:

Mouse EL-4 lymphoblast cells ( $1 \times 10^6$ ) were cultured for 3 days in DMEM supplemented with 10% FBS and stimulated with 10 ug/mL PMA and 10 ug/mL PHA. An aliquot of the cell culture supernatant was removed and assayed for levels of mouse TGF- $\beta$ 1.

Condition	(ng/mL)
Unstimulated	1.1
Stimulated	3.9

	Sample1	Sample2	Sample3	Sample4
SP2/O cell culture supernatant	648 pg/mL	846 pg/mL	1,044 pg/mL	1,236 pg/mL

## 9.5 Sensitivity

The minimum detectable dose of mouse TGF-beta1 is 16.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## 9.6 Linearity

To assess the linearity of the assay, mouse serum and cell culture supernatant samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The mouse serum was initially diluted 1:30. Cell culture supernatant was initially diluted 1:2)

		Mouse serum	Cell culture supernatant
1:2	Average% of Expected	86	91
	Range (%)	83-90	86-97
1:4	Average% of Expected	102	101
	Range (%)	95-108	93-110
1:8	Average% of Expected	102	102
	Range (%)	92-111	86-116
1:16	Average% of Expected	112	105
	Range (%)	102-123	86-116

## 10. References

1. Siegel, P.M.et al. (2003) Nat Rev Cancer 3: 807-21.
2. Bierie, B. et al. (2006) Nat Rev Cancer 6: 506-20.
3. Tian, M. et al. (2009) Future Oncol 5: 259-71.
4. Priyadarshi S. et al.(2013) 28: 2490-7.