Rat TGF-beta1 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE20010

Size: 96T

Sensitivity: 3.4 pg/mL

Range: 31.25 - 2000 pg/mL

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

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

Table of content page

1. Background 3

2. Principle 3

3. Required Materials 3

4. Kit Components and Storage 4

5. Safety Notes 4

6. Sample Collection and Storage 4

7. Regent Preparation 4

8. Assay Procedure Summary 7

9. Validation Data 8

9.1 Standard curve 8

9.2 Precision 8

9.3 Recovery 9

9.4 Sample values 9

9.5 Sensitivity 9

9.6 Linearity 10

10. References 10

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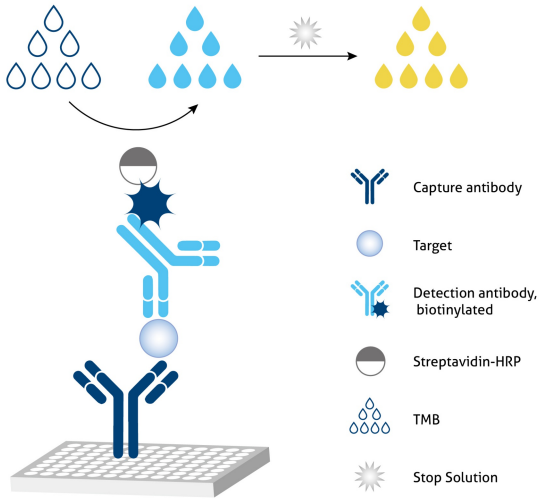
1. Background

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. TGF-beta appears to promote late stage progression and metastasis in some cancers.

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.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
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	2 bottles	
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. Regent 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).

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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.

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for activated cell culture supernatant; 1:250, 1:500 is recommended for activated serum or plasma.

To activate latent TGF-beta1 to the immunoreactive form, prepare the following solutionsfor 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/ 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 acidifcation and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it iswithin 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 supernatants, 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 acidifed sample by adding 20 µL of 1.2 N NaOH	Neutralize the acidifed sample by adding 25 µL of 1.2 N NaOH
Mix well.	Mix well.
Prior to the assay, dilutethe activated sample with Sample Diluent PT 1-ef. See the following data for suggested dilutions.	Prior to the assay, dilute the activated sample with Sample Diluent PT 1-ef. See the following data for suggested dilutions.
The concentration read of the standard curve must be multiplied by the dilution factor, 1.4.	The concentration read of the standard curve must be multiplied by the appropriate dilution factors, 2.

Do not activate the kit standards. The kit standards contain active recombinant TGF-beta 1 .

Activated serum and plasma samples may be stored for up to 24 hours at 2-8 °C before use. Activated cell Aculture supernatant sample must be assayed immediately after activation. Do not freeze activated samples.

NB: This ELISA kit is cross-reactive with bovine, porcine, equine, and caprine. Therefore, the culture medium should not contain serum components associated with the above species.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 1-ef in protein standard

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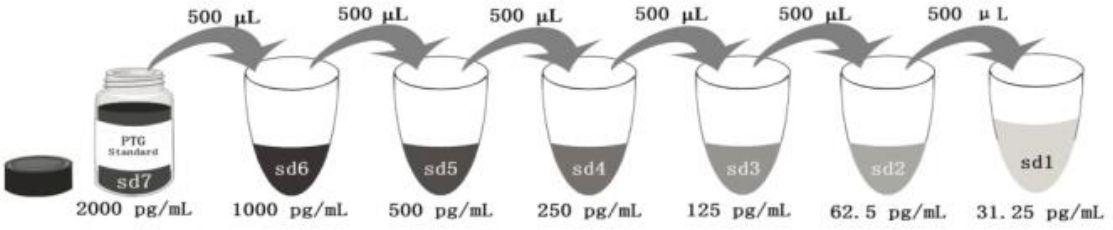

Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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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 Preparation7.2) 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 Preparation7.3) 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.				

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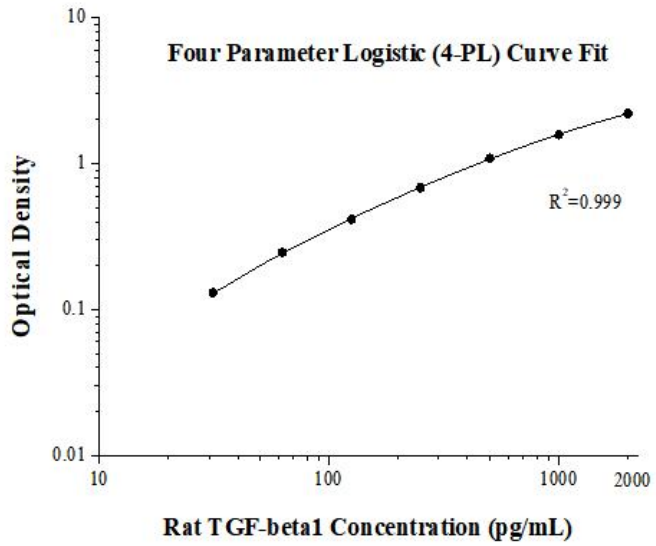
9. Validation Data

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	0.055 0.049	0.052	-
31.25	0.18 0.183	0.182	0.13
62.5	0.301 0.295	0.298	0.246
125	0.46 0.473	0.467	0.415
250	0.739 0.737	0.738	0.686
500	1.154 1.129	1.142	1.09
1000	1.659 1.609	1.634	1.582
2000	2.266 2.255	2.261	2.209

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	43.6	3.3	7.5
2	20	245.3	11.1	4.5
3	20	1027.5	53.2	5.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	55.5	4.5	8.1
2	24	251.8	23.0	9.1
3	24	1079.7	94.4	8.7

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9.3 Recovery

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

Sample Type		Average% of Expected	Range (%)
Rat serum	1:1000	101	93-106
	1:2000	97	81-114
Cell culture supernatant	1:5.6	98	78-116
	1:11.2	101	81-126

9.4 Sample values

Sample Type	Mean (ng/mL)	Range (ng/mL)
Rat serum (n=16)	74.4	21.1-130

Cell culture supernatant - Supernatant of rat spleen cells were cultured in DMEM supplemented with 10% fetal bonive serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured 3 days. Aliquots of the cell culture supernatant was removed and assayed for levels of rat TGF-beta1, the value is 684.4 pg/mL.

9.5 Sensitivity

The minimum detectable dose of rat TGF-beta1 is 3.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, three samples were were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.(The rat serum and plasma activated samples were initially diluted 1:120)

		Rat serum	Cell culture supernatant
1:2	Average% of Expected	98	100
	Range (%)	88-107	-
1:4	Average% of Expected	100	110
	Range (%)	-	96-120
1:8	Average% of Expected	88	102
	Range (%)	78-100	96-107
1:16	Average% of Expected	89	113
	Range (%)	78-109	93-121

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.

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