Speedy™ Human TGF beta 1 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50233

Size: 96T

Sensitivity: 0.4 pg/mL

Range: 31.25 -2000 pg/mL

Usage: For the quantitative detection of human TGF beta 1 concentrations in serum, plasma and urine.

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

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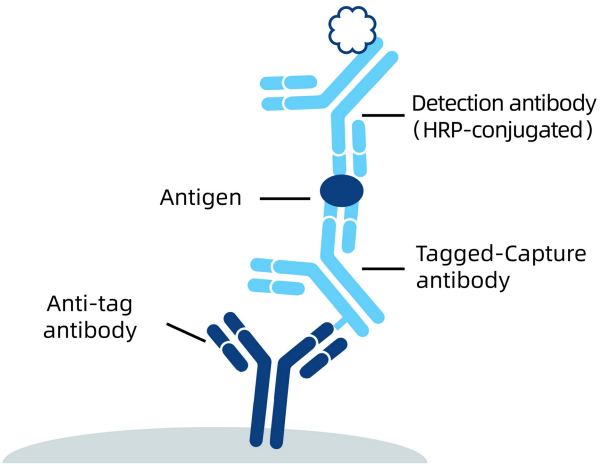
1. Background

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



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the 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. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (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	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	1 bottle	
Detection Diluent - 15 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 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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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 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

7.3 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 beta 1 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/ 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 acidifcation 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.

Urine	Human serum and plasma
To 100 µL of urine, 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/0.5M HEPES.	Neutralize the acidifed sample by adding 25 µL of 1.2 N NaOH/0.5M HEPES.
Mix well Mix well.	Mix well Mix well.
Prior to the assay, dilute the activated sample with Sample Diluent PT 4B1. See the following datas for suggested dilutions.	Prior to the assay, dilute the activated sample with Sample Diluent PT 4B1. See the following datas for suggested dilutions.
The dilution factor is 1.4.	The dilution factor is 2.

Recommended Dilution for different sample types: 1:80 or 1:160 is recommended for activated human serum and plasma; 1:2 or 1:4 is activated recommended for urine.

Note: The concentration reads must be multiplied by the dilution factors and recommended dilution.

7.5 Standard Serial Dilution:

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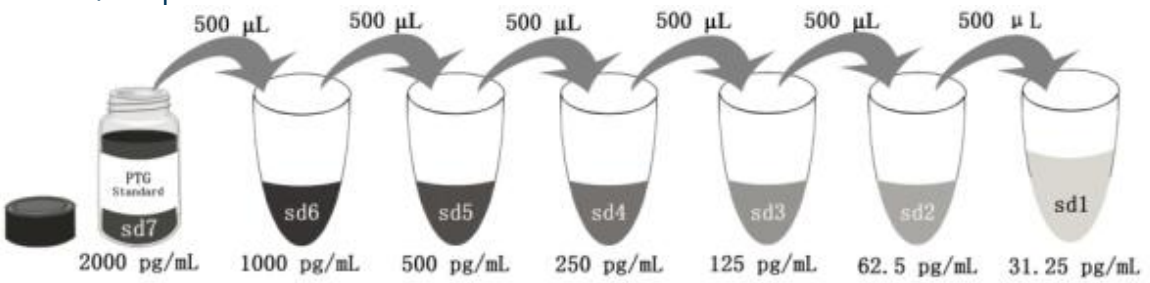
Add 2 mL Sample Diluent PT 4B1 in protein standard.



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 4B1	**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, HRP-conjugated antibody 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 Preset the layout of the microplate, including control group, standard group and sample group, 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 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

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 1× 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 TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 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, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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Add 50 µL standard or sample to appropriate wells

↓

Add 50 µL antibody cocktail solution (1x) to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

↓

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

↓

Add 100 µL TMB substrate solution to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

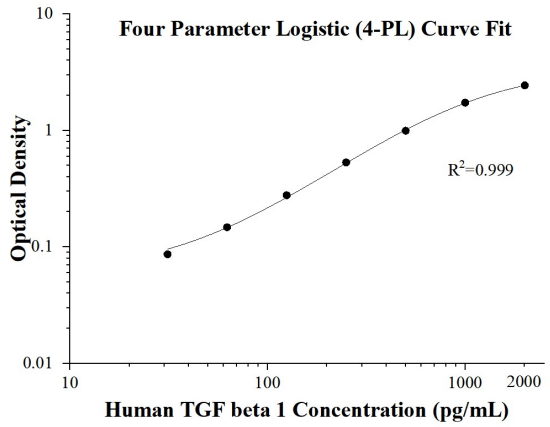
↓

Add 100 µL Stop Solution to each well and Read OD immediately

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	0.0282 0.0298	0.0290	-
31.25	0.1122 0.1188	0.1155	0.0865
62.5	0.1775 0.176	0.1768	0.1478
125	0.2921 0.3217	0.3069	0.2779
250	0.5433 0.5771	0.5602	0.5312
500	1.0394 1.0046	1.0220	0.9930
1000	1.7701 1.7538	1.7620	1.7330
2000	2.4636 2.4651	2.4644	2.4354

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	1,030.1	22.0	2.1
2	8	258.2	6.4	2.5
3	8	129.0	3.8	2.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	1,036.5	20.7	2.0
2	16	260.8	11.9	4.6
3	16	127.0	4.6	3.6

9.3 Recovery

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

Sample Type		Average% of Expected	Range (%)
Human serum	1:640	97	95-99
	1:1,280	96	94-98
Urine	1:2.8	79	74-85

9.4 Sample values

Human serum/Urine - Human serum and urine samples were evaluated for the presence of human TGF beta 1 in this assay.

Sample Type	Mean (**ng/mL**)	Range (**ng/mL**)
Human serum (n=16)	67.9	7.3-120.8

Sample Type	Mean (pg/mL)	%Detectable	Range (pg/mL)
Urine (n=8)	48.3	37.5	ND-59.7

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human TGF beta 1 is 0.4 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, urinet samples were spiked with high concentrations of human TGF beta 1 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Human serum samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The activated human serum was initially diluted 1:40.)

		Human serum	Urine
1:2	Average% of Expected	100	97
	Range (%)	-	91-103
1:4	Average% of Expected	108	94
	Range (%)	107-109	91-96
1:8	Average% of Expected	118	103
	Range (%)	114-121	102-105
1:16	Average% of Expected	124	107
	Range (%)	120-128	105-108

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9.7 Specificity

This assay recognizes natural and recombinant human TGF beta 1 .

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Recombinant mouse:	Recombinant rat:
BMP-3	TGF-β RI	TGF beta 1
GDF-15	TGF-β RII	
BMP-5	BMPR-IA	
BMP-10	BMP-3	
BMP-7	BMPR-IB	
Agrin		
TGF-β RI		
TGF-α		
BMPR-IB		
Activin A		
BMP8A		
BMP6		
BMP8B		
BMP-2		
BMP-4		
TGF-β1		
BMPR-1A		
GDF-11		

A sample containing 50 ng/mL of the recombinant mouse TGF beta 1 reads as 50 pg/mL (0.10% cross-reactivity).

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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