Mammalian TGF beta1 ELISA Instructions

Cat:EH0012

Content

	CAT	Volume
❶ CP (Coated Plate)	EH0012CP	96 well
❷ S (Standard)	EH0012S1	2 vial
❸ AC (Acid Buffer)	EH0001AC	1.8 ml/vial
❹ AL (Alkali Buffer)	EH0001AL	1.8 ml/vial
❺ 100×DA (Detect Antibody)	EH0012DA	50 μl/vial
❻ SH (Streptavidin-HRP)	ESH01	12 ml/bottle
❼ AB (Assay Buffer 1×)	EAB01	15 ml/bottle
❽ TS (TMB Substrate)	ETS01	12 ml/bottle
❾ SS (Stop Solution)	ESS01	12 ml/bottle
❿ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
⓫ SF (Sealer Film)	ESF01	6 pieces

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

REAGENT PREPARATION

Washing Buffer (1×) Preparation

Pour entire contents (50 ml) of the Washing Buffer Concentrate (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

Detect Antibody (1×) Preparation

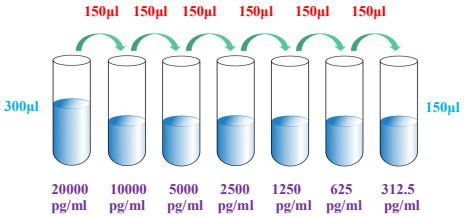
Calculate the volume of Detect Antibody (1×) required by the experiment; use the AB (Assay Buffer) to dilute 100× DA by 100 times.

REAGENT PREPARATION

Standard Curve Preparation

Reconstitute mammalian TGF beta1 Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Allow the standard to reconstitute for at least 15 minutes. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 20 ng/ml).

Pipette 150 μl of **AB (Assay Buffer)** into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. **AB (Assay Buffer)** serves as the zero standard (0 pg/ml).



Sample Activation Procedure

To activate latent TGF-β1 to immunoreactive TGF-β1 detectable, follow the activation procedure outlined below.

Sample pretreatment

To 20 µL of cell culture supernate、urine、tissue grinding liquid、serum and plasma, add 20 µL of **AC (Acid Buffer)**, mix well. Incubate 10 minutes at room temperature. Neutralize the acidified sample by adding 20 µL of **AL (Alkali Buffer)** and mix well. Prior to the assay, dilute the activated sample with **AB (Assay Buffer)**. The concentration read off the standard curve must be multiplied by the appropriate dilution factor.

Sample dilution

Human serum samples require a 20-fold dilution in **AB (Assay Buffer)**. A suggested 20-fold dilution is 10 µL of activated sample + 190 µL of **AB (Assay Buffer)** (final dilution factor of sample is 60).

Cell culture supernate samples (human and non-human) tested neat, but may require dilution if high endogenous levels are present (final dilution factor of sample is 3 if tested neat).

Human urine samples tested neat (final dilution factor of sample is 3).

Tissue grinding liquid samples tested neat (final dilution factor of sample is 3).

Human plasma samples require a 8-fold dilution in **AB (Assay Buffer)**. A suggested 8-fold dilution is 25 µL of activated sample + 175 µL of **AB (Assay Buffer)** (final dilution factor of sample is 24).

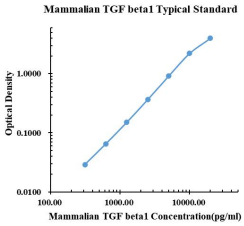
Mouse and rat serum/ plasma samples require a 50-fold dilution in **AB (Assay Buffer)**. A suggested 50-fold dilution is 10 µL of activated sample + 490 µL of **AB (Assay Buffer)** (final dilution factor of sample

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50 µl of **AB** (Assay Buffer) to each well.
4. Add 50 µl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 µl of **1× DA** (Detect Antibody) to each well.
6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hours on a microplate **shaker** set at 500 rpm.
7. Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.
8. Add 100 µl of **SH** (Streptavidin-HRP) to each well.
9. Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.
10. Repeat aspiration/**wash** as in step 7.
11. Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
12. Add 100 µl of **SS** (Stop Solution) to each well.
13. Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



pg/ml	O.D.		Average	Corrected
0.00	0.0245	0.0213	0.0229	
312.50	0.0520	0.0516	0.0518	0.0289
625.00	0.0852	0.0902	0.0877	0.0648
1250.00	0.1688	0.1769	0.1729	0.1500
2500.00	0.3794	0.3927	0.3861	0.3632
5000.00	0.9181	0.9431	0.9306	0.9077
10000.00	2.2980	2.1540	2.2260	2.2031
20000.00	4.0220	4.0026	4.0123	3.9894

PRECISION

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

Inter-assay Precision (Precision between assays)

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (pg/ml)	195.0	1090.8	3619.9	237.3	1154.4	3711.7
Standard Deviation	10.3	67.7	202.6	16.3	67.5	144.7
Coefficient of Variation (%)	5.3	6.2	5.6	6.8	5.8	3.9

SENSITIVITY

The minimum detectable dose (MDD) of mammalian TGF beta1 is typically less than 33.32 pg/ml.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating

RECOVERY

The spike recovery was evaluated by spiking 3 levels of mammalian TGF beta1 into health serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 88% to 105% with an overall mean recovery of 99%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of mammalian TGF beta1 in serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 95% to 104% with an overall mean recovery of 100%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers, thirty samples from apparently healthy mice and thirty samples from apparently healthy rats were evaluated for the presence of mammalian TGF beta1 in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Human Serum	30	35.3-74.7	100	58.4
Mouse Serum	30	117.4-139.7	100	126.7
Rat Serum	30	50.4-78.5	100	62.4

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.

CALIBRATION

The NIBSC/WHO British Standard for human leukocyte mammalian TGF beta1 89/514 was evaluated in this kit. To convert sample values obtained with the mammalian TGF beta1 kit to relative approximate NIBSC units, use the equation below:

NIBSC/WHO (89/514) approxmate value (U/ml) = 0.016 × mammalian TGF beta1 value (pg/ml)