

Mammal TGF beta1 ELISA Instructions

Cat: EH0012

Content

	CAT	Volume
① CP (Coated Plate)	EH0012CP	96 well
② S (Standard)	EH0012S1	2 vial
③ AC (Acid Buffer)	EH0001AC	1 vial
④ AL (Alkali Buffer)	EH0001AL	1 vial
⑤ 100×DA (Detect Antibody)	EH0012DA	1 vial
⑥ SH (Streptavidin-HRP)	ESH01	12 ml/bottle
⑦ AB (Assay Buffer 1×)	EAB01	15 ml/bottle
⑧ TS (TMB Substrate)	ETS01	12 ml/bottle
⑨ SS (Stop Solution)	ESS01	12 ml/bottle
⑩ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
⑪ 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°C.

Detect Antibody (1×) Preparation

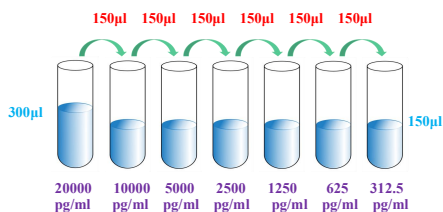
Calculate the volume of Detect Antibody (1×) required by the experiment; use the **AB (Assay Buffer)** to dilute **100× DA** by 100 times.

REAGENT PREPARATION

Standard Curve Preparation

Reconstitute Mammal 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- β 1 to immunoreactive TGF- β 1 detectable, follow the activation procedure outlined below.

■ Sample pretreatment

To 20 μ L of cell culture supernate, urine, serum or plasma, add 20 μ L of **AC (Acid Buffer)**, mix well. Incubate 10 minutes at room temperature. Neutralize the acidified sample by adding 20 μ L 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 μ L of activated sample + 190 μ L 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).

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

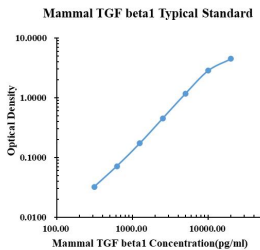
Mouse and rat serum/ plasma samples require a 50-fold dilution in **AB (Assay Buffer)**. A suggested 50-fold dilution is 10 μ L of activated sample + 490 μ L of **AB (Assay Buffer)** (final dilution factor of sample is 150).

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 μ L of **AB** (Assay Buffer) to each well.
- 4 Add 50 μ L of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
- 5 Add 50 μ L of **1 \times DA** (Detect Antibody) to each well.
- 6 Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) 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 μ L). 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 μ L of **SH** (Streptavidin-HRP) to each well.
- 9 Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) for 30 min on a microplate **shaker** set at 500 rpm.
- 10 Repeat aspiration/**wash** as in step 7.
- 11 Add 100 μ L of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
- 12 Add 100 μ L 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	0.0219	0.0270	0.0245
312.50	0.0561	0.0568	0.0565
625.00	0.0973	0.0945	0.0959
1250.00	0.1937	0.1982	0.1960
2500.00	0.4737	0.4704	0.4721
5000.00	1.2210	1.1640	1.1925
10000.00	2.8550	2.8810	2.8680
20000.00	4.4672	4.5023	4.4848

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		
	S1	S2	S3	S1	S2	S3
Sample Number	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 mammal 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 the corresponding concentration.

RECOVERY

The spike recovery was evaluated by spiking 3 levels of mammal 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 mammal 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 were evaluated for the presence of mammal TGF beta1 in this assay. No medical histories were available for the donors.

Sample Matrix	Number of Sample	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	35.3-74.7	100	58.4

Serum/Plasma – Thirty samples from apparently healthy mice were evaluated for the presence of mammal TGF beta1 in this assay. No medical histories were available for the donors

Sample Matrix	Number of Sample	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	117.4-139.7	100	126.7

Serum/Plasma – Thirty samples from apparently healthy rats were evaluated for the presence of mammal TGF beta1 in this assay. No medical histories were available for the donors.

Sample Matrix	Number of Sample	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
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 H/M/R TGF beta1 89/514 was evaluated in this kit. To convert sample values obtained with the mammal TGF beta1 kit to relative approximate NIBSC units, use the equation below:

NIBSC/WHO (89/514) approximate value (U/ml) = $0.016 \times \text{mammal TGF beta1 value (pg/ml)}$