Human GITR/TNFRSF18 ELISA Kit

Instructions for Use | Cat. No.: AEH0095

1. Content

Component	CAT	Volume
CP (Coated Plate)	EH0095CP	96 well
S (Standard)	EH0095S,S1~S7,S0	9 vial
DA (Detect Antibody)	EH0095DA	6 ml/bottle
SH (Streptavidin-HRP)	ESH01	12 ml/bottle
AB (Assay Buffer 1×)	EAB01	12 ml/bottle
TS (TMB Substrate)	ETS01	12 ml/bottle
SS (Stop Solution)	ESS01	12 ml/bottle
WB (Wash Buffer 10×)	EWB01	50 ml/bottle
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.

2. 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.

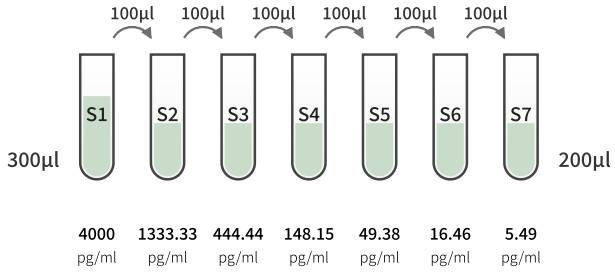
Standard Curve Preparation

S1 to S7 and S0 is ready to use for serum and plasma.

Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc.

Urine sample use AB (Assay Buffer) prepare standard curve.

The human GITR/TNFRSF18 Standard EH0095S 40000 pg/ml 30 µl + 270 µl SPB serves as the high standard (4000 pg/ml). Pipette 200 µl of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).

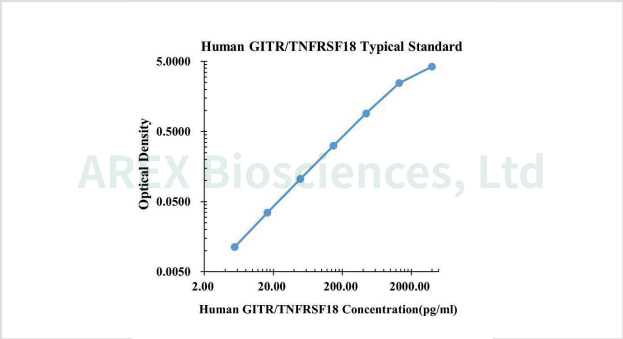


3. Assay Procedure

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 or 10 µl of Standard or sample per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 µl of **DA** (Detect Antibody) to each well.
6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) for 1 hour 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°C) 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.

4. Typical Data



(pg/ml)	O.D.	Average	Corrected
0.00	0.0145 / 0.0146	0.0146	-
5.49	0.0260 / 0.0256	0.0258	0.0113
16.46	0.0492 / 0.0497	0.0495	0.0349
49.38	0.1206 / 0.1200	0.1203	0.1058
148.15	0.3372 / 0.3175	0.3274	0.3128
444.44	0.9332 / 0.9130	0.9231	0.9086
1333.33	2.4945 / 2.4639	2.4792	2.4647
4000.00	4.1397 / 4.2936	4.2167	4.2021

5. Sensitivity

The minimum detectable dose (MDD) of human GITR/TNFRSF18 is typically less than 0.07 pg/ml (50 µl of sample volume) or 1.20 pg/ml (10 µl of sample volume).

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.

6. Precision

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested six times on one plate to assess intra-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average(pg/ml)	1410.0	535.3	111.0	1556.0	680.1	136.7
Standard Deviation	55.5	10.1	2.4	102.5	32.1	9.1
Coefficient of Variation(%)	3.9	1.9	2.2	6.6	4.7	6.7

7. Recovery

The spike recovery was evaluated by spiking 3 levels of human GITR/TNFRSF18 into healthy human serum sample.

The un-spiked serum was used as blank in this experiment. The recovery ranged from 84% to 111% with an overall mean recovery of 98%.

8. Linearity

To assess the linearity of the assay, five samples were spiked with high concentration of GITR/TNFRSF18 in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 93% to 109% with an overall mean recovery of 105%.

9. Sample Values

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of human GITR/TNFRSF18 in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (pg/ml)	Detectable %	Mean of Detectable (pg/ml)
Serum	30	n.d.-1.19	75	0.47

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