antibodies -online.com





TNFSF18 ELISA Kit



Image



\sim							
	1//	$rac{1}{2}$	`\/	L	Р	1	٨

Quantity:	96 tests
Target:	TNFSF18
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	4-1000 pg/mL
Minimum Detection Limit:	4 pg/mL
Application:	ELISA
Product Details	
Purpose:	Mouse GITR Ligand (TNFSF18) ELISA Kit for cell culture supernatants, plasma, and serum
	samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30L,
	CD30, CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-
	CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5,
	IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin,
	Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-
	3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1 alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha,
	TNF RI, TNF RII, TPO, VCAM-1, VEGF.

Product Details

Sensitivity:	< 4 pg/mL
Characteristics:	Strip plates and additional reagents allow for use in multiple experiments
	Quantitative protein detection
	Establishes normal range
	The best products for confirmation of antibody array data
Components:	Pre-Coated 96-well Strip Microplate
	Wash Buffer
	Stop Solution
	Assay Diluent(s)
	Lyophilized Standard
	Biotinylated Detection Antibody
	Streptavidin-Conjugated HRP
	TMB One-Step Substrate
Material not included:	Distilled or deionized water
	 Precision pipettes to deliver 2 μL to 1 μL volumes
	 Adjustable 1-25 μL pipettes for reagent preparation
	 100 μL and 1 liter graduated cylinders
	Tubes to prepare standard and sample dilutions
	Absorbent paper
	 Microplate reader capable of measuring absorbance at 450nm
	 Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	TNFSF18
Alternative Name:	GITR Ligand (TNFSF18 Products)
Gene ID:	240873
UniProt:	Q7TS55

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 fold	
Sample Volume:	100 μL	
Plate:	Pre-coated Pre-coated	
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well.	

- 3. Incubate 2.5 h at RT or O/N at 4 °C.
- 4. Add 100 µL of prepared biotin antibody to each well.
- 5. Incubate 1 h at RT.
- 6. Add 100 μL of prepared Streptavidin solution to each well.
- 7. Incubate 45 min at RT.
- 8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
- 9. Incubate 30 min at RT.
- 10. Add 50 µL of Stop Solution to each well.
- 11. Read at 450 nm immediately.

Reagent Preparation:

- 1. Bring all reagents and samples to room temperature (18 25 °C) before use.
- 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants. Suggested dilution for normal serum/plasma: 2 fold*. * Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
- 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
- 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 μ L Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 20 μ L GITR Ligand standard (50 ng/mL) from the vial of Item C, into a tube with 980 μ L Assay Diluent A or 1x Assay Diluent B to prepare a 1,000 pg/mL standard solution. Pipette 300 μ L Assay Diluent A or 1x Assay Diluent B into each tube. Use the 1,000 pg/mL standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 20 μ L standard + 980 μ L 200 μ L 200 myl 1,000 400 160 64 25.6 10.24 4.10 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL
- 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
- 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μ L of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.
- 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add

 $50~\mu L$ of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 200-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure:

- 1. Bring all reagents and samples to room temperature (18 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
- 2. Add 100 μ L of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.
- 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 4. Add 100 μ L of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
- 5. Discard the solution. Repeat the wash as in step
- $6. \text{ Add } 100 \ \mu\text{L}$ of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
- 7. Discard the solution. Repeat the wash as in step
- 8. Add 100 μ L of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
- 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

<u>Typical Data:</u> These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Mouse GITR-L concentration (pg/mL) 1 10 100 1000 10000 0 D = 450 nm 0.01 0.1 1 10 Assay Diluent B Mouse GITR-L concentration (pg/mL) 1 10 100 1000 10000 0 D = <math>450 nm 0.01 0.1 1 10

<u>Sensitivity:</u> The minimum detectable dose of GITR Ligand is typically less than 4 pg/mL.

<u>Recovery:</u> Recovery was determined by spiking various levels mouse GITR Ligand into mouse serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 99.40 91-107 Plasma 98.14 91-103 Cell culture media 105.3 97-113

Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 118.9

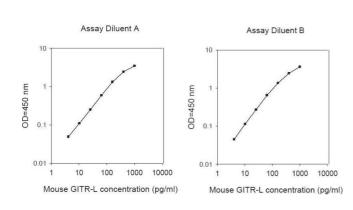
Application Details

	123.8 101.8 Range (%) 111-127 115-132 94-110 1:4 Average % of Expected 124.5 140.0 80.92
	Range (%) 117-133 131-148 73-89
	Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Images



ELISA

Image 1.