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# **TNFSF4 ELISA Kit**



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Overview		
Quantity:	96 tests	
Target:	TNFSF4	
Reactivity:	Mouse	
Method Type:	Sandwich ELISA	
Detection Range:	15.6 pg/mL - 1000 pg/mL	
Minimum Detection Limit:	15.6 pg/mL	
Application:	ELISA	
Product Details		
Purpose:	For the quantitative determination of mouse tumor necrosis factor ligand superfamily member 4 (TNFSF4) concentrations in serum, plasma, cell culture supernates, tissue homogenates.	
Sample Type:	Cell Culture Supernatant, Plasma, Serum, Tissue Homogenate	
Analytical Method:	Quantitative	
Detection Method:	Colorimetric	
Specificity:	This assay has high sensitivity and excellent specificity for detection of mouse TNFSF4. No significant cross-reactivity or interference between mouse TNFSF4 and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between mouse TNFSF4 and all the analogues, therefore, cross reaction may still exist.	
Sensitivity:	3.9 pg/mL	

## **Product Details**

Components:

- Assay plate
- Standard
- HRP-avidin (100 x concentrate)
- Biotin-antibody (100 x concentrate)
- · Sample Diluent
- · HRP-avidin Diluent
- · Biotin-antibody Diluent
- Wash Buffer (25 x concentrate)
- · TMB Substrate
- · Stop Solution
- · Adhesive Strip

# Target Details

Target:	TNFSF4	
Alternative Name:	tumor necrosis factor (ligand) superfamily, member 4 (TNFSF4 Products)	
Background:	Abbreviation: TNFSF4 Alias: CD134L, CD252, GP34, OX-40L, OX40L, TXGP1, CD134 ligand OX40 antigen ligand tax-transcriptionally activated glycoprotein 1 (34kD)	
UniProt:	P43488	
Pathways:	Cellular Response to Molecule of Bacterial Origin, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Activated T Cell Proliferation, Cancer Immune Checkpoints	

## **Application Details**

Application Notes:	Optimal working dilution should be determined by the investigator.
Sample Volume:	100 μL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	<ol> <li>Prepare reagents, samples and standards as instructed.</li> <li>Add 100 μL standard or sample to each well. Incubate 2 hours at 37 °C.</li> <li>Remove the liquid of each well, don't wash.</li> <li>Add 100 μL Biotin-antibody (1x) to each well. Incubate 1 hour at 37 °C.</li> <li>Aspirate and wash 3 times.</li> </ol>
	6. Add 100 µL HRP-avidin (1x) to each well. Incubate 1 hour at 37 °C

- 7. Aspirate and wash 5 times.
- 8. Add 90  $\mu$ L of TMB Substrate to each well. Incubate for 15-30 minutes at 37 °C. Protect from light.
- 9. Add 50  $\mu$ L Stop Solution to each well. Read at 450 nm within 5 minutes.

### Reagent Preparation:

- 1. Biotin-antibody (1x) Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of Biotin-antibody + 990  $\mu$ L of Biotin-antibody Diluent.
- 2. HRP-avidin (1x) Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of HRP-avidin + 990  $\mu$ L of HRP-avidin Diluent.
- 3. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
- 4. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (1000 pg/mL). Sample Diluent serves as the zero standard (0 pg/mL).

#### Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- · Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents.
   Contaminated water or container for reagent preparation will influence the detection result.

#### Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The

# **Application Details**

	recommended dilution factor is for reference only.  • Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).	
Assay Precision:	Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known	
	concentration were tested twenty times on one plate to assess.	
	Inter-assay Precision (Precision between assays): CV%<10% Three samples of known	
	concentration were tested in twenty assays to assess.	
Restrictions:	For Research Use only	
Handling		
Storage:	4 °C,-20 °C	
Storage Comment:	Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. May be stored fo	
	up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and	
	avoid the damp. Standard May be stored for up to 1 month at 2 - 8° C. If Biotin-antibody don't	
	make recent use, better keep it store at HRP-avidin -20°C. Biotin-antibody Diluent Opened kit	
	HRP-avidin Diluent Sample May be stored for up to 1 month at 2 - 8°C. Diluent Wash Buffer	
	TMB Substrate Stop Solution *Provided this is within the expiration date of the kit.	
Expiry Date:	6 months	