Catalog Number: CUBEK01824

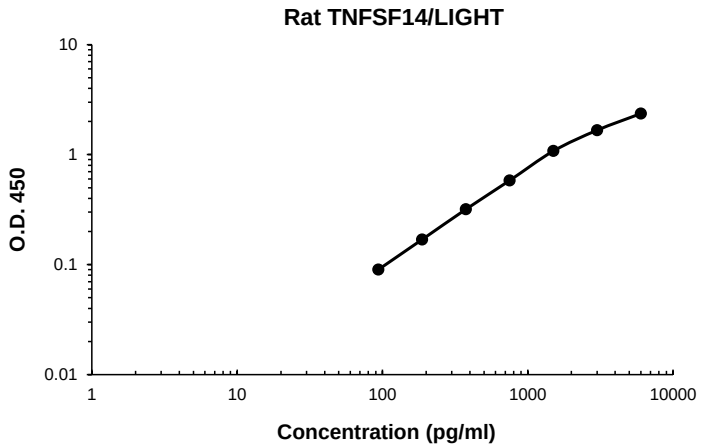
Size: 12 X 8-well Strips (96T)

For quantitative detection of rat TNFSF14 in cell culture supernatants, serum and plasma (heparin, EDTA).

Typical Data:

(TMB reaction: 20 - 25 minutes)

Concentration (pg/ml)	0	93.7	187.5	375.0	750.0	1500.0	3000.0	6000.0
O.D.	0.011	0.09	0.169	0.318	0.581	1.077	1.665	2.359



Detection Range:	Sensitivity:	Sample Types:
93.7 pg/ml - 6000 pg/ml	< 10 pg/ml	cell culture supernatants, serum and plasma (heparin, EDTA)

Specificity: Natural and recombinant rat TNFSF14. There is no detectable cross-reactivity with other relevant proteins.

Storage: Store at 4˚C for 1 month, at -20˚C for up to longer storage. Avoid multiple freeze/thaw cycles (Shipped with blue ice.)

Principle

The rat TNFSF14/LIGHT ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TNFSF14 has been pre-coated onto a 96-well plate. Standards and test samples are added to the wells. A biotinylated detection monoclonal antibody specific for TNFSF14 is added, followed by washing with PBS or TBS buffer. An Avidin-Biotin-Peroxidase Complex is added and the unbound conjugates are washed away with PBS or TBS buffer. HRP substrate (TMB) is used to visualize the HRP enzymatic reaction. The HRP-TMB reaction initially produces a blue color that changes yellow upon adding the acidic stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the amount of rat TNFSF14/LIGHT within the sample that is captured by the plate.

Notice Before Application

Please read the following instructions before starting the experiment.

1. In order to familiarize yourself with the protocol and determine appropriate sample dilutions, an initial pilot experiment using a few samples is recommended.
2. The TMB Color Developing agent is colorless and transparent. Before using, please contact us if it is not clear.
3. Prior to using the Kit, spin tubes briefly to ensure no liquid remains in the tube caps.
4. Duplicates are recommended for both standard and sample testing.
5. Do not let the 96-well plate dry out. If this occurs, the components will become inactive.
6. To avoid cross contamination, do not re-use pipette tips.
7. Use reagents from the same batches during processing.
8. Pre-warm the diluted ABC and TMB solution at 37°C for 30 min prior to use. Using pre-warmed solutions will help reduce any marginal effects due to temperature differences across the plate.

Kit Components

Catalog number	Description	Quantity
	96-well plate precoated with anti-rat TNFSF14/LIGHT antibody	1
CUBEK00501	Lyophilized recombinant rat TNFSF14/LIGHT standard	2 Tubes
	Biotinylated anti-rat TNFSF14/LIGHT antibody	130 µl (dilution 1:100)
	Avidin-Biotin-Peroxidase Complex (ABC)	130 µl (dilution 1:100)
CUBEK00504	Sample dilution buffer	30 ml
CUBEK00505	Antibody dilution buffer	12 ml
CUBEK00506	ABC dilution buffer	12 ml
	TMB color developing agent	10 ml
	TMB stop solution	10 ml
	Adhesive cover	4

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. If working with a large number of samples, we recommend using a multi-channel pipette.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral 0.01 M PBS or 0.01 M TBS).
   - Preparation of 0.01 M **TBS:** Add 1.2 g Tris, 8.5 g Nacl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L.
   - Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L.

Protocol Summary

1. Add samples and standards. Incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.0 1 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 20 - 25 min.
5. Add TMB stop solution and read.

Preparation

1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.

Cell culture supernatants: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the standard curve. Dilute the sample using the provided dilution buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the dilution buffer.**

High target protein concentration (10X Upper Detection Range - >100X Upper Detection Range). The working dilution is 1:100. I.e. Add 1 µl sample into 99 µl sample dilution buffer.

Medium target protein concentration (2X Upper Detection Range - 10X Upper Detection Range). The working dilution is 1:10. I.e. Add 10 µl sample into 90 µl sample dilution buffer.

Low target protein concentration (2X Lower Detection Range - 2X Upper Detection Range). The working dilution is 1:2. I.e. Add 50 µl sample to 50 µl sample dilution buffer.

Very Low target protein concentration (0 – 2X Lower Detection Range). No dilution necessary, or the working dilution is 1:2.

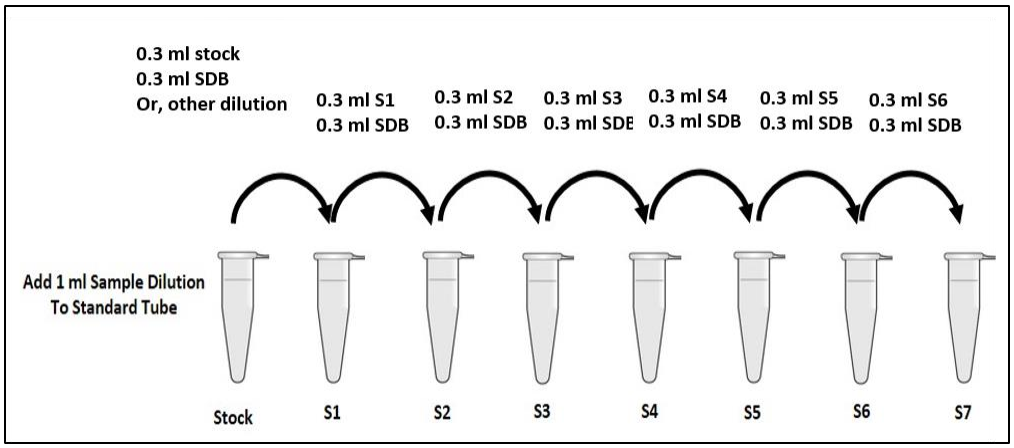
3. Reagent Preparation and Storage

A. Reconstitute TNFSF14 standard solution no more than 2 hours prior to the experiment.

Prepare standards at the following concentrations: 6000 pg/ml, 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.7 pg/m .

1. Label 7 tubes from: 6000 pg/ml, 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.7 pg/ml
2. Create a standard stock solution by adding 1 ml of sample dilution buffer to one tube of standard. Let sit for 10 min at room temp and mix thoroughly. Use one tube of standard per experiment.

3. It is recommended that most standards be created by performing 1:2 serial dilutions. However, an initial, non-1:2 dilution may be necessary. For example: 1) If the standard stock solution concentration is 10,000 pg/ml (10 ng/ml) and the highest required standard is 500 pg/ml, create the 500 pg/ml standard by adding 50 µl of standard stock solution into 950 µl of sample dilution buffer (mix thoroughly). 2) If the standard concentration is 20,000 pg/ml and the highest required standard is 8000 pg/ml, create the 8000 pg/ml standard by adding 400 µl of standard stock solution into 600 µl of sample dilution buffer (mix thoroughly).
4. Perform all other standard dilutions by aliquoting 0.3 ml of standard into 0.3 ml of sample dilution buffer. Mix each standard thoroughly as it is prepared.



Note: The standard solutions are best used within 2 hours. The standard stock solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-Rat TNFSF14/LIGHT antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1 - 0.2 ml more than total volume)
2. Biotinylated anti-Rat TNFSF14/LIGHT antibody should be diluted in 1:100 with the antibody dilution buffer and mixed thoroughly. (I.e. Add 1 µl Biotinylated anti-Rat TNFSF14/LIGHT antibody to 99 µl antibody dilution buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1 - 0.2 ml more than total volume)
2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (I.e. Add 1 µl ABC to 99 µl ABC dilution buffer.)

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, mix thoroughly to ensure a homogenous solution. A standard detection curve should be prepared for each experiment. The user will decide sample dilutions.

1. Aliquot 0.1 ml per well of the 6000 pg/ml, 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.7 pg/ml solutions into the pre-coated 96-well plate. Add 0.1 ml of the sample dilution buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each standard solution and each sample be

measured in duplicate.

2. Seal the plate with a new adhesive cover provided and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-rat TNFSF14/LIGHT antibody working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 60 min.
5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS. Allow wash to sit in the wells for 1 minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1 ml of prepared ABC working solution into each well, seal the plate with a new adhesive cover provided and incubate at 37°C for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 - 2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well, seal the plate with a new adhesive cover provided and incubate at 37°C in dark for 20 - 25 min (**Note:** For reference only, the optimal incubation time should be determined by end user. Shades of blue can be seen in the wells with the four most concentrated rat TNFSF14/LIGHT standard solutions; the other wells may show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat TNFSF14/LIGHT concentration of the samples can be interpolated from the standard curve. **Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.