

Mouse Anti-Human TRAIL ELISA Set

<u>Cat No.</u>	<u>Form</u>	<u>Quantity</u>
11045-01	ELISA Set	1

For Research Use Only

Intended Use

This set is specifically designed for quantitative determination of human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) concentrations in cell culture supernatant, plasma and serum.

Background

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of cytokines. This type II transmembrane protein is expressed by several cytotoxic immune cells, including natural killer cells and activated T cells, and is selective for tumorigenic and virally infected cells^{1,2}. TRAIL can bind to DR4 and DR5 receptors on target cells which facilitates recruitment of numerous signaling proteins to form a death-inducing signaling complex. This signaling complex is then believed to propagate the apoptotic signal through the recruitment and activation of Caspase-8^{3,4}.

Numerous studies have identified TRAIL as a potent apoptotic factor in a variety of human cancer cell lines, but this cytokine does not appear to affect normal cell lines⁵. Therefore, the selectivity and anti-tumorigenic properties of TRAIL make it an intriguing and potentially therapeutic agent in the treatment of cancer.

Principle of Assay

This assay incorporates a quantitative sandwich enzyme immunoassay technique. Unlabeled monoclonal antibody specific for human TRAIL is first coated onto 96 well microplate(s). Standards and samples are then added to the wells and any TRAIL present is captured by the immobilized antibody. After a wash to remove unbound material, biotin-labeled anti-human TRAIL detection antibody is added to the wells, followed by a horseradish peroxidase-labeled anti-biotin antibody incubation. After another wash, TMB substrate solution is added that will result in a blue color proportional to the amount of bound TRAIL. Color development is then quenched and intensity is measured at 450nm.

Research Applications

Enzyme-Linked-Immunosorbent-Assay (ELISA) Characterization

To ensure lot-to-lot consistency, each batch of product is tested by ELISA to conform to the characteristics of a standard reference reagent.

Assay Kit Components

- 0.3ml TRAIL Capture Antibody: unlabeled, 200X
- 0.12ml TRAIL Detection Antibody: biotin-conjugated, 500X
- 0.3ml Revealing Antibody: HRP-conjugated, 200X
- 2 vials Standard: lyophilized recombinant human TRAIL, 20ng/vial
- 15ml Coating Buffer (5X)
- 300ml Assay Diluent (1X)
- 300ml Washing Buffer (20X)
- 60ml Standard/Sample Diluent (1X)
- 60mL TMB one component microwell substrate
- 60mL TMB Stop Solution
- 5 x 96-well ELISA plates

Sample Collection and Storage

Cell Culture Supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using citrate, EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

All reagents should be diluted immediately prior to use. Do not add sodium azide to Assay Diluent as it inhibits the activity of horseradish-peroxidase. It is recommended that all samples, controls and standards be assayed in duplicate or triplicate.

- Dilute 5X Coating Buffer to 1X with distilled (DI) water. A suggested dilution (for one plate) consists of 2.2mL 5X Coating Buffer with 8.8mL of DI water.

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- Dilute unlabeled human TRAIL specific Capture Antibody 200-fold with 1X Coating Buffer. A suggested dilution (for one plate) consists of 55 μ L Capture Antibody with 10.945mL 1X Coating Buffer.
- Dilute 20X Wash Buffer to 1X with DI water. A suggested dilution consists of 50mL 20X Wash Buffer with 950mL of DI water.
- Reconstitute 1 vial lyophilized human TRAIL Standard by adding 0.1ml DI water to 1 vial (200ng/mL) and gently vortex. Next, dilute TRAIL Standard 100-fold by adding 5 μ L 200ng/mL Standard to 495 μ L Standard Diluent to yield a final concentration of 2000pg/mL and allow the standard to equilibrate for at least 15 minutes. Aliquot any unused standard into polypropylene vials and store at -80°C.
- Dilute biotin-labeled human TRAIL specific Detection Antibody 500-fold with 1X Assay Diluent. A suggest dilution (for one plate) consists of 22 μ L Detection Antibody with 10.978mL 1X Assay Diluent.
- Dilute horseradish peroxidase-labeled Revealing Antibody 200-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 55 μ L Revealing Antibody with 10.945mL 1X Assay Diluent.

Assay Procedure

1. Collect samples and prepare reagents as directed previously.
2. Add 100 μ L of diluted Capture Antibody solution to all wells of a provided 96-well plate. Incubate at 4°C overnight.
3. Wash entire plate 4 times with 1X Wash Buffer.
4. Add 300 μ L of 1X Assay Diluent per well to block non-specific binding and reduce background. Incubate at room temperature for 30 minutes.
5. Wash plate as in Step 3.
6. Prepare standard dilution by adding 100 μ L/well of appropriate dilution to the plate. To do this, perform six two-fold serial dilutions of the 2000pg/mL top standard either within the plate or in separate tubes. To dilute within the plate, add 200 μ L of 2000pg/mL human TRAIL Standard to row A and 100 μ L Standard/Sample Diluent to rows B through H. Perform serial dilution by taking 100 μ L standard from row A and mix with row B. Then take 100 μ L from row B and add it to row C and continue for each subsequent row. Therefore, the final human TRAIL standard concentrations will be 2000pg/mL, 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL and 31.3pg/mL. Standard/Sample Diluent alone serves as the zero standards for row H (0pg/mL). For test samples, dilute two-fold with Standard/Sample Diluent and add 100 μ L/well. Incubate at room temperature for 2 hours.
7. Wash plate as in Step 3.
8. Add 100 μ L of diluted biotin-labeled Detection Antibody solution to each well and incubate at room temperature for 1 hour.
9. Wash plate as in Step 3.
10. Add 100 μ L of diluted HRP-labeled Revealing Antibody solution to each well and incubate at room temperature for 30 minutes.
11. Wash plate 6 times with 1X Wash Buffer. For this final wash, soak wells in Wash Buffer for 10 seconds for each wash. This will help minimize background.
12. Add 100 μ L of TMB Substrate Solution to each well. Incubate at room temperature for 10-15 minutes. The Substrate Solution should turn blue in color.
13. Add 50 μ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells turns green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

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- Determine the optical density of each well within 30 minutes using a microplate reader set to 450 nm.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on log-log graph paper, with human TRAIL concentration on the x-axis and the mean absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the test sample human TRAIL concentration, find the mean absorbance value of the test sample on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the TRAIL concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred. If a test sample's O.D. value falls outside the linear portion of the standard curve, the test sample should be reanalyzed at an alternate dilution as appropriate.

Specificity

This assay recognizes recombinant and native human TRAIL. No cross-reactivity or interference with other human proteins was observed.

Sensitivity

The minimum detectable concentration of human TRAIL is 1.2pg/mL.

Calibration

The human TRAIL standard in this set was calibrated against the NIBSC/WHO International Standard 04/166.

1.0ng Southern Biotech human TRAIL standard = 0.978ng of NIBSC/WHO human TRAIL standard.

Recovery

The recovery of human TRAIL spiked to levels throughout the range of the assay in serum and cell culture supernatant was evaluated by the set. At least 85% of the TRAIL was recovered from serum and 95% from cell culture supernatant.

Trouble Shooting

General technical hints:

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- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- During incubation steps, shaking the plates may increase sensitivity.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue upon addition.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color development in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that turn green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Poor Signal

- Avoid sodium azide in wash buffers as it inhibits the enzymatic activity of HRP
- Verify that appropriate antibody pairs were used
- Inadequate reagent volumes added to wells
- Incorrect incubation times and/or temperature

Poor Standard Curve or Precision

- Improper standard handling and/or dilution
- Inadequate mixing of reagents
- Inadequate aspiration and/or washing of wells

References

1. Zamai, L. et al. (1998). *J. Exp. Med.* **188**:2375-80.
2. Jin, Z. and El-Deiry, W.S. (2006). *Mol. Cell. Biol.* **26**:8136-48.
3. Thorburn, A. (2004). *Cell. Sign.* **16**:139-44.
4. Micheau, O. and Tschopp, J. (2003). *Cell.* **114**:181-90.
5. Nagane, M. et al. (2001). *Apop.* **6**:191-7.

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