CELL APPLICATIONS, INC.

# Human TRAIL ELISA Kit

Cat. No. CL0532
96-wells

| | |
|---|---|
| **Target Protein Species** | Human |
| **Range** | 15.6pg/ml-1000pg/ml |
| **Specificity** | No detectable cross-reactivity with any other cytokine. |

## COMPONENTS

| Kit Component | Amount |
|---|---|
| 96-well plate precoated with anti-human TRAIL antibody | 1 Plate |
| Protein Standard: Lyophilized recombinant human TRAIL | 2 tubes, 10 ng/tube |
| Sample Diluent Buffer | 30 ml |
| Biotinylated Antibody (Anti-human TRAIL) | 130 µl (100x) |
| Antibody Diluent Buffer | 12ml |
| Avidin-Biotin-Peroxidase Complex (ABC) Solution | 130 µl (100x) |
| ABC Diluent Buffer | 12 ml |
| Tetramethylbenzidine (TMB) Color Developing Agent | 10 ml |
| TMB Stop Solution | 10 ml |

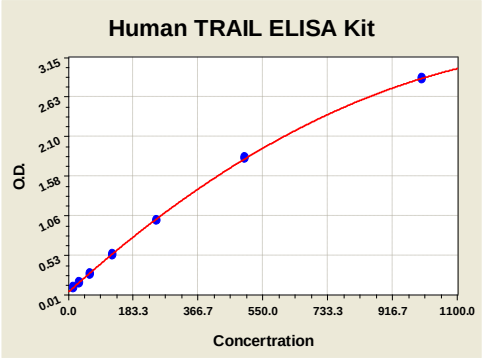
**Washing Buffer (not provided): TBS or PBS**

0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 900ml $H_2O$ and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

0.01M PBS: Add 8.5g sodium chloride, 1.4g $Na_2HPO_4$ and 0.2g $NaH_2PO_4$ to 900ml distilled $H_2O$ and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

**Storage**

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.




| X | pg/ml | 0.0 | 15.6 | 31.3 | 62.5 | 125 | 250 | 500 | 1000 |
|---|---|---|---|---|---|---|---|---|---|
| Y | O.D.450 | 0.061 | 0.117 | 0.184 | 0.299 | 0.546 | 1.001 | 1.833 | 2.872 |

**Figure 1: TRAIL Standard Curve.** Using the Human TRAIL ELISA Kit, O.D. data was graphed against TRAIL protein concentration. The TMB reaction was incubated at 37°C for 15 min.

## BACKGROUND

TNF-related apoptosis-inducing ligand (TRAIL) or Apo2 ligand ( Apo2L) is one of several members of the TNF gene superfamily that induce apoptosis through engagement of death receptors. TRAIL/ Apo2L is unusual as compared to any other cytokine as it interacts with a complex system of receptors: two pro-apoptotic death receptors and three anti-apoptotic decoys. This protein has generated tremendous excitement as a potential tumor-specific cancer therapeutic because, as a stable soluble trimer, it selectively induces apoptosis in many transformed cells but not in normal cells. Transcriptional activation of TRAIL/ Apo2L by interferons (IFNs) through specific regulatory elements in its promoter, and possibly by a number of other cytokines, reveals its possible involvement in the activation of natural killer cells, cytotoxic T lymphocytes, and dendritic cells.[1]

The interaction of TRAIL with its two death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2) is the initial step in TRAIL-induced apoptosis. The binding of TRAIL leads to trimerization of the death receptors and activation of receptor-mediated death pathway. The activated death receptors recruit and activate an adaptor protein called FADD through interactions between the death domain (DD) on the death receptors and FADD. The death effector domain (DED) of FADD recruits and activates caspase-8, leading to the formation of the death-inducing signaling complex (DISC). In type I cells, the presence of activated caspase-8, is sufficient to induce activation of one or more effector caspases, which then act on final death substrates in apoptosis. However, in type II cells, even a small amount of activated caspase-8, although not enough to activate the effector caspases, is sufficient to trigger a mitochondria-dependent apoptotic amplification loop by activating Bid, which induces the accumulation of Bax in mitochondria, the release of cytochrome c from mitochondria, the activation of caspase-9, -3, and -7, and finally, programmed cell death.[2] In addition to inducing apoptosis by caspase-8 recruitment through FADD, TRAIL binding to its receptors also leads to activation of the NF-kB. TRAIL death receptors, like TNFR1, activate NF- NF-kB through the TNFR1-associated death domain protein (TRADD). Activated TRADD recruits the DD-containing protein RIP and TRAF2, leading to activation of the NF-kB pathway. In TNFR1 signaling, TRADD is believed to be upstream of FADD. However, in TRAIL signaling, TRADD may be mediated by FADD, because TRADD recruitment to the DISC is observed only in the presence of FADD.[3]

## ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethylbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

**NOTES:**

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

**References**


1. Aggarwal, B.B. et al: Vitam. Horm. 67:453-83, 2004
2. Srivastava, R.K.: Neoplasia 3:535-46, 2001
3. Yang, X. & Thiele, C.J.: Cancer Lett. 197:137-43, 2003

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## PROTOCOL

### I. Plate Washing

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.

### II. Preparation of Test Samples

**Test Sample Processing**

- **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
- **Serum**: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min.
- **Plasma**: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

**Sample Dilution Guideline**

Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

| Target Protein Concentration Range | Sample Working Dilution | Sample Vol. | Diluent Buffer Vol. |
|---|---|---|---|
| 10-100 ng/ml | 1:100 | 1 µl | 99 µl |
| 1-10 ng/ml | 1:10 | 10 µl | 90 µl |
| 156-10,000 pg/ml | 1:2 | 50 µl | 50 µl |
| ≤156 pg/ml | n/a | 100µl | n/a |

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

### III. Preparation of Reagents

**Reconstitution of the Standard**

The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.

1. 10,000pg/ml of human TRAIL standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. 5000pg/ml→156pg/ml of human TRAIL standard solutions: Label 6 Eppendorf tubes with 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000pg/ml TRAIL standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

**Preparation of Biotinylated Antibody Working Solution**

The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

**Preparation of the 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: 0.1ml/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.

### IV. ELISA

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, they must be mixed completely and evenly. A standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of target protein amount in samples.

1. Aliquot 0.1ml per well of the 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml human TRAIL standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Blank well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline"** for details. We recommend that each human TRAIL standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover 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 0.1ml of biotinylated anti-human TRAIL antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M 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.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 15-30 min (shades of blue can be seen in the wells with the four most concentrated human TRAIL standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution..

### V. Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)
- Plot the standard curve:
  Plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The target protein concentration in samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.

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