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# ProductInformation

**TRAIL ELISA, Human**

Product Number **CS0510**
Storage Temperature 2-8 °C

## Technical Bulletin

### Product Description

TRAIL ELISA is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) for quantitative determination of TRAIL protein in cell lysates. A monoclonal antibody specific for TRAIL (regardless of phosphorylation state) has been coated onto the wells of the multiwell plate strips provided. TRAIL standard dilutions, control specimens, and unknown samples are pipetted into these wells. During the first incubation, the TRAIL antigen binds to the immobilized (capture) antibody. After incubation, the excess reagents are washed away and an Anti-Human TRAIL Biotin Conjugate, is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized TRAIL. After removal of excess biotin conjugate, Streptavidin- peroxidase (SAV-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove excess Streptavidin-peroxidase, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of TRAIL present in the original specimen. The optical density measured at 450 nm in the multiwell plate reader is used to calculate the concentration of TRAIL.

TRAIL ELISA is designed to detect and quantify the level of TRAIL protein in serum, plasma, cell extracts, buffered solution, or cell culture medium.

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is a recently identified member of the TNF gene superfamily. Five different receptors have been identified for TRAIL. Two receptors, DR4 and DR5, are transmembrane proteins containing death domain similar to FAS and other TNF family receptors. Two other receptors, DcR1 and DcR2, act like decoy proteins for TRAIL binding because they lack the death domain. TRAIL can also bind, though weakly, to osteoprotegrin (OPG), a soluble receptor, which plays a role in osteoclastogenesis. TRAIL induces apoptosis in various tumor cell lines, whereas most primary cells seem to be resistant. TRAIL-mediated apoptosis occurs following its binding to DR4 or DR5 receptors. The mechanism of apoptosis involves activation of caspase-8 and subsequent activation of effector caspases. Also, NF-κB and JNK activation play a role in the TRAIL signaling pathway.

TRAIL expression is detectable in many normal organs and tissues. Several studies suggest that TRAIL may play a physiological role by contributing to immune privilege, normal cellular development, and inhibition of autoimmune responses. The expression of TRAIL is upregulated in activated T cells, B cells, NK cells, monocytes and macrophages. LPS activation stimulates the release of soluble TRAIL from monocytes and macrophages, suggesting a role of TRAIL in their cytotoxic/phagocytic function. Also, TRAIL expression is increased in transformed cell lines and various diseases including cancer and autoimmune disorders. TRAIL may also be involved in activation-induced T cell death during HIV infection. Certain anti-cancer agents also upregulate TRAIL and TRAIL receptor expression on tumor cells, thus sensitizing cells to apoptosis.

### Reagents

- **TRAIL Standard, Lyophilized, 2 vials, Product . No. T 4325 -** purified recombinant hTRAIL expressed in *E. coli*.. Refer to vial label for quantity and reconstitution volume.
- **Standard Diluent Buffer, 25 mL, Product No. S 5694,** contains sodium azide as preservative.
- **Monoclonal-Anti-Human TRAIL-Coated 96 well plate, 1EA, Product No. T 4450 -** A plate using break-apart strips coated with monoclonal antibody specific for full-length TRAIL (regardless of phosphorylation state).
- **Anti-Human TRAIL Biotin-Conjugate, 11 mL, Product No. T 4200** A detection antibody, produced in rabbit. Contains sodium azide. Ready to use.

- **Streptavidin-Peroxidase (SAV-HRP) Concentrate (100X), 1 vial, Product No. S 7819 -** contains 3.3 mM thymol and 50% glycerol, viscous. *See Reagent Preparation for handling, dilution and storage instructions.*
- **Streptavidin-HRP Diluent, 25 mL, Product No. H 8912 -** contains 3.3 mM thymol. Ready to use.
- **Wash Buffer concentrate, 25X, 100 mL, Product No. W 2639 -** *See Reagent Preparation for handling, dilution and storage instructions*
- **Stabilized Chromogen, Tetramethylbenzidine (TMB), 25 mL, Product No. S 3318** – Avoid prolonged exposure to light. Avoid exposure to metal. Ready to use.
- **Stop Solution, 25 mL, Product No. S 2818** – Ready to use.
- **Plate Covers, Adhesive strips, 3 each, Product No. P 4870**

**Reagents and Equipment required but not provided**

- Multiwell plate reader capable of readings at 450 nm.
- Calibrated adjustable precision pipettes for volumes between 5 µL and 1,000 µL.
- Cell extraction buffer (see recommended extraction procedure).
- Deionized or distilled water.
- Plate washer (optional), use squirt bottle, manifold dispenser, etc.
- Glass or plastic 1.0 – 1.5 mL tubes for diluting and aliquoting standard.
- Absorbent paper towels to blot the plate.
- Calibrated beakers and graduated cylinders in various sizes.
- Vortex mixer.
- Graph paper: linear, log-log, or semi-log, as desired.

**Precautions and Disclaimer**

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

Sample Preparation

- Samples of choice – extracts of cell lysates
- Samples should be frozen if not analyzed shortly after collection.
- Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

Cell Extraction Buffer

10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM $Na_3VO_4$
1% Triton® X-100
10% glycerol
0.1% SDS
0.5% deoxycholate

1 mM PMSF (stock is 0.3 M in DMSO) *PMSF is very unstable and must be added prior to use, even if added previously*.

Protease inhibitor cocktail (Sigma Product No. P 2714)

Add 250 µl of reconstituted cocktail per 5 mL of Cell Extraction Buffer.

Stable for 2-3 weeks at 4 °C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at –20 °C.

*Thaw on ice*. *Add the protease inhibitors just before use.*

Procedure for Extraction of Proteins from Cells

This protocol has been successfully applied to several cell lines of human origin. Researchers may use the procedures that work best in their hands. They will have to assay their lysates for the satisfactory extraction and/or phosphorylation.

Protocol for Cell Extraction

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at –70 °C and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10-minute intervals.

5. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of TRAIL. Under these conditions, use of a 1:10 dilution of cell extract with Standard Diluent Buffer (See **Assay Method**) is sufficient for the detection of TRAIL.
6. Other extraction methods can be employed, but the initial dilution to be made must be optimized. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4 °C.
7. Aliquot the clear lysate to clean microcentrifuge tubes

Before assay: extracted cell lysate samples containing protein should be diluted with Standard Diluent Buffer at least 1:10. This dilution is necessary to reduce the matrix effect of the cell extraction buffer.

Reagent Preparation

**Standard**

1. Reconstitute TRAIL Standard with *Standard Diluent Buffer* to 3000 pg/mL. Refer to standard vial label for instructions.
2. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 3000 pg/mL. Use standard within 1 hour of reconstitution.
3. Prepare serial standard dilutions as follows

| Tube # | Standard Buffer | Standard from tube #: | Final pg/mL |
|---|---|---|---|
| 1 | Reconstitute according to label instructions | | 3000 pg/mL |
| 2 | 0.300 mL | 0.300 mL (1) | 1500 pg/mL |
| 3 | 0.300 mL | 0.300 mL (2) | 750 pg/mL |
| 4 | 0.300 mL | 0.300 mL (3) | 375 pg/mL |
| 5 | 0.300 mL | 0.300 mL (4) | 187.5 pg/mL |
| 6 | 0.300 mL | 0.300 mL (5) | 93.7 pg/mL |
| 7 | 0.300 mL | 0.300 mL (6) | 46.8 pg/mL |
| 8 | 0.300 mL | - | 0 pg/mL |

4. Remaining reconstituted standard should be discarded or frozen at –70 °C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.

**Streptavidin-Peroxidase**

*Note:* The *Streptavidin-HRP* 100X concentrate is in 50% glycerol. This solution is viscous. To ensure accurate dilution:

1. Equilibrate to room temperature, mix gently, pipette slowly.
2. Remove excess concentrate solution from pipette tip with clean absorbent paper.
3. Within 1 hour of use, dilute 10 µL of this 100X concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as *Streptavidin*--HRP Working Solution.
4. Return the unused concentrate to the refrigerator
5. For more strips use the following amounts:

| # of 8 well strips | SAV-HRP Concentrate µL | Diluent mL |
|---|---|---|
| 2 | 20 | 2 |
| 4 | 40 | 4 |
| 6 | 60 | 6 |
| 8 | 80 | 8 |
| 10 | 100 | 10 |
| 12 | 120 | 12 |

**Wash Buffer**

1. Equilibrate to room temperature and mix to redissolve any precipitated salts.
2. Mix 1 volume Wash Buffer Concentrate 25X + 24 volumes of deionized water
3. Label as *Working Wash Buffer.*
4. Store both the concentrate and the Working Wash Buffer in the refrigerator. Use within 14 days.

**Storage/Stability**

All components of this kit are stable at 2-8 °C. Any unused reconstituted standard should be discarded or frozen at –70 °C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Refer to the Certificate of Analysis for kit shelf life. To obtain C of A go to www.sigma-aldrich.com

**Procedure**

Precautions

- 20-30 minutes before use equilibrate kit and all reagents to room temperature (15-30 °C).
- Use only the coated 96 well capture plate provided with the kit
- Multiwell plate: equilibrate to room temperature in unopened foil bag. Remove desired number of strips, reseal the bag and refrigerate at 2-8 °C to maintain plate integrity.
- When not in use all kit components should be refrigerated.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay

- Maintain a consistent order of component and reagent addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.
- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Do not use reagents after the kit expiration date.
- Standards and samples can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Read absorbances within 2 hours of assay completion.

Washing directions

- The purpose of washing is to remove unbound proteins and other non-specific parts of lysate.
- Incomplete washing will adversely affect the assay and render false results.
- Use only Wash Buffer provided in kit.
- Washing may be performed using automated washer, manifold pipette or squirt bottle.
- Wash cycle four times, blotting as dry as possible after the 4th wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.
- It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

Assay Procedure

**TRAIL ELISA Assay Summary**

**1. 100 µL of Standards or Samples**
**(samples diluted 1:10 or higher in Standard Diluent Buffer)**

**Incubate 2 hours at RT**
**aspirate and wash 4x**

**2. Add 100 µL Anti-Human TRAIL Biotin-Conjugate**

**Incubate 1 hour at RT.**
**aspirate and wash 4x**

**3. Add 100 µL Streptavidin-HRP**

**Incubate 30 min at RT.**
**aspirate and wash 4x**

**4. Add 100 µL Stabilized Chromogen**

**Incubate 30 minutes at RT**
***(in the dark)*.**

**5. Add 100 µL of Stop Solution**

**Read at 450nm.**

**Total Assay Time - 4 hours**

- Determine the number of wells for the assay run, including 2 zero wells, 2 chromogen blank wells, 14 standard dilution wells and 2 wells for each sample to be assayed.
- Remove appropriate number of multiwell strips and return the unused strips to the pouch. Reseal pouch

1st incubation

a Add 100 µL Standard Diluent to zero wells.
b Add 100 µL standards, samples or controls to the appropriate wells.
c Samples prepared in cell extraction buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10 µL sample plus 90 µL buffer). *The dilutions should be optimized for each assay.*
d Cell culture supernatants or buffered solutions; dilute 1:2 in *Standard Diluent Buffer* (50 µL buffer + 50 µL sample).
e Tap gently on the plate to mix, cover with Plate Cover and incubate 2 hours at room temperature.
f Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells for a total of 4 times following washing instructions

2nd incubation

a Add 100 µL Anti-TRAIL detection antibody (Biotin conjugate) to all wells (except chromogen blanks).
b Tap gently on the plate to mix, cover with Plate Cover and incubate 1 hour at room temperature.
c Wash wells for a total of 4 times following washing instructions.
d After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

3rd incubation
a Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blanks.
b Cover with Plate Cover and incubate 30 minutes at room temperature.
c Wash wells for a total of 4 times following washing instructions.
d After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

Substrate incubation
a Add 100 µL of Stabilized Chromogen into all wells. *The liquid in the wells will begin to turn blue.*
b Do not cover the plate
c Incubate approximately 30 minutes at room temperature in the dark (*place plate in a drawer or cabinet).*

Note: *If your multiwell plate reader does not register optical density (OD) above 2.0, incubate only 20-25 minutes.*

Stop reaction
a Add 100 µL of Stop Solution to each well. This stops the reaction
b Tap gently to mix. *The solution will turn yellow.*

Absorbance reading
a Any commercially available multiwell plate reader capable of reading at OD 450 nm may be used.
b Blank the plate reader against the Chromogen Blank wells (contain Chromogen and Stop Solution).

Read the absorbance of the entire plate at 450 nm within 2 hours after addition of Stop Solution

**Results**
1. The results may be calculated using any immunoassay software package
2. The four-parameter algorithm provides the best curve fit.
3. If the software program is not readily available, the concentrations of TRAIL may be calculated manually.
4. Calculate the Average Net OD (average reading of 2 wells) for each standard dilution and samples as follows:
5. Average Net OD = Average Bound OD – Average Chromogen Blank OD
6. On graph paper plot the Average Net OD of standard dilutions against the standard concentration (pg/mL) of TRAIL. Draw the best curve through these points to construct the standard curve.
7. The TRAIL concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
8. Multiply the values obtained for the samples by dilution factor of each sample.
9. Samples producing signals higher than the 3000 pg/mL standard should be further diluted and assayed again.

**Product Profile**

Typical Results
The standard curve below is for illustration only and **should not be used** to calculate results in your assay. Run standard curve in each assay.

| Standard HuTRAIL (pg/mL) | Optical Density 450 nm |
|---|---|
| 0 | 0.177 |
| 46.8 | 0.243 |
| 93.7 | 0.302 |
| 187.5 | 0.408 |
| 375 | 0.615 |
| 750 | 0.952 |
| 1500 | 1.698 |
| 3000 | 2.944 |

Limitations
- Do not extrapolate the standard curve beyond the 3000 pg/mL standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated.
- The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of cell extract and brain homogenate samples have not been thoroughly investigated.
- The rate of degradation of native TRAIL in various matrices has not been investigated.

**Performance characteristics**

Sensitivity
The minimum detectable dose of TRAIL is <20 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision

1. Intra-Assay Precision

Samples of known concentration were assayed in replicates of 16 to determine precision within an assay.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 275 | 1066 | 2293 |
| Standard Deviation (SD) | 13.3 | 54.6 | 68.3 |
| % Coefficient of Variation | 4.8 | 5.1 | 2.9 |

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

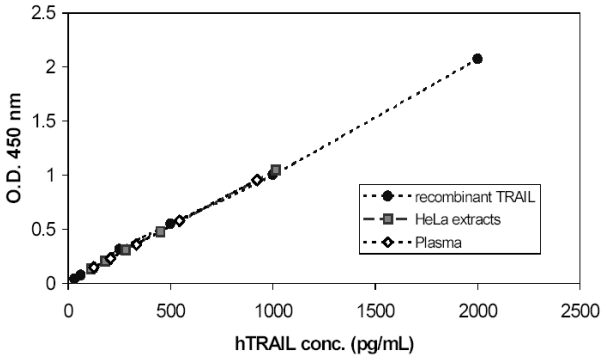
| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 282 | 1121 | 2230 |
| Standard Deviation (SD) | 19.7 | 101.2 | 143.4 |
| Coefficient of Variation % | 6.9 | 9.0 | 6.4 |

Recovery

The recovery of hTRAIL added to human serum averaged 85%, while the recovery of hTRAIL added to tissue culture medium containing 10% fetal calf serum averaged 105%. The recovery of hTRAIL added to citrate or heparin plasma averaged 77.1 and 98.4%, respectively. EDTA plasma yielded poor recovery and is not recommended.

Parallelism

Natural hTRAIL from plasma or HeLa cell lysate were serially diluted in *Standard Diluent Buffer.* The optical density of each dilution was plotted against the standard curve. Parallelism between the natural and recombinant proteins was demonstrated by the figure below and indicated that the standard accurately reflects natural hTRAIL content in samples.




**Parallelism between recombinant and natural hTRAIL**

Linearity of Dilution

Human serum and tissue culture medium containing 10% fetal calf serum were spiked with hTRAIL and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Specificity

Buffered solutions of a panel of substances at 80,000 pg/mL were assayed with the human TRAIL ELISA. The following substances were tested and found to have no cross-reactivity:
human DR4 (TRAIL receptor 1), DR5 (TRAIL receptor 2), basic FGF, IFN-γ, VEGF, TGF-α, TGF-β, FasL; mouse G-CSF, TNF-α; Bovine FGF.

Expected Values

Seven sera and ten plasma (heparin) samples from healthy individuals were evaluated in this assay. The values for sera ranged from 100 to 1800 pg/mL. The values for plasma (heparin) ranged from 300 to 1000 pg/mL. A limited number of commercially available pooled serum samples measured 100 to 500 pg/mL. A limited number of cell culture supernatants ranged from 0 to 200 pg/mL.
Cell extracts of several human cell lines were also tested (diluted 1:10 in *Standard Diluent*). The values (after correction for dilution) ranged from 0.45 to 15 ng/mL at 1 mg/mL of total protein.

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