cellsciences.com

Human TRAIL/CD253 ELISA KIT

Catalog No. CDK058A **Quantity:** 1 x 96 tests

Size:	*1 plate (pre-coated)*
Specificity:	Recognizes both native and recombinant soluble human TRAIL
Range:	93.75 pg/ml - 3000 pg/ml
Sensitivity:	<64 pg/ml
Incubation	3 hr 45 min
Sample Types:	Serum Plasma Cell culture supernatant
Sample Size:	100 µl
Crossreactivity:	No cross reactivity with other human soluble molecules.

1. INTENDED USE

The Cell Sciences® Human TRAIL/Apo2L/CD253 ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of TRAIL in supernatants, buffered solutions or serum and plasma samples. This assay will recognize both natural and recombinant human TRAIL. **This kit has been configured for research use only.**
Background: TNF-Related Apoptosis-Inducing Ligand (TRAIL), is a member of the tumor necrosis factor (TNF) family, also designated as Apo-2 ligand (Apo-2L) and CD253. TRAIL is a 281-amino acid protein, related most closely to Fas/Apo-1 ligand. It is expressed at cell surface as a transmembrane protein with an extracellular region and a homotrimeric subunit structure and also exists as a soluble form
TRAIL interacts with several receptors: two pro-apoptotic death receptors DR4 (TRAIL R1) and DR5 (TRAIL R2) and three anti-apoptotic decoy receptors DcR1 (TRAIL R3), DcR2 (TRAIL R4) and osteoprotegrin (OPG). Soluble TRAIL induces apoptosis through these death receptors in many transformed cells (lymphoid as well as non-lymphoid tumor cell lines).
Because of its role in apoptosis and its stable trimer, TRAIL was described as a potential tumor-specific cancer therapeutic.

2. PRINCIPLE OF THE METHOD

A capture antibody highly specific for TRAIL has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of TRAIL samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-TRAIL secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of TRAIL present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of TRAIL in any sample tested.

Cell Sciences ®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

3. REAGENTS PROVIDED AND RECONSTITUTION

Reagents (Store@2-8°C)	Quantity 1 x 96 well kit	Reconstitution
P: 96 well microtiter strip plate	1	Ready to use. (Pre-coated)
A: Plastic plate covers	2	n/a
B: TRAIL Standard: 3000 pg/ml	2	Reconstitute as directed on the vial. (see Assay preparation, section 8)
C: Standard Diluent (Buffer)	1 (25 ml)	Ready-to-use.
D: TRAIL Control	2	Reconstitute as directed on the vial (see reagent preparation, section 8)
E: Biotinylated anti-TRAIL	1 (0.4 ml)	Dilute in Biotinylated Antibody Diluent. (see Assay preparation, section 8)
F: Biotinylated Antibody Diluent	1 (7 ml)	Ready to use.
G: Streptavidin-HRP	2 (5 µl)	Add 0.5 ml of HRP diluent prior to use. (see Assay preparation, section 8)
H: HRP Diluent	1 (23 ml)	Ready to use.
I: Wash Buffer	1 (10 ml)	200x Concentrate dilute in distilled water. (see Assay preparation, section 8)
J: TMB Substrate	1 (11 ml)	Ready to use.
K: H_2SO_4 stop reagent	1 (11 ml)	Ready to use.

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 630 nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single channel micropipettes with disposable tips
- 50-300 µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. STORAGE INSTRUCTIONS

Store kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Wash buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Standard diluent Buffer 1X: Once prepared, store at 2-8°C for up to 1 week.

Reconstituted Standard / Control: Once prepared, use immediately and do not store.

Diluted Biotinylated Anti-TRAIL: Once prepared, use immediately and do not store.

Diluted Streptavidin-HRP: Once prepared, use immediately and do not store.

6. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid repeated freeze-thaw cycles and stored frozen at -80°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

7. SAFETY AND PRECAUTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g.CDC/NIH Health manual: “Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min. of the washing of the microtiter plate.

8. ASSAY PREPARATION

Bring all reagents to room temperature before use.

8.1 Assay Design

Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example plate layout (example shown for a 6 point standard curve)

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	3000	3000										
B	1500	1500										
C	750	750										
D	375	375										
E	187.5	187.5										
F	93.75	93.75										
G	zero	zero										
H	control	control										

All remaining empty wells can be used to test samples in duplicate.

8.2 Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2˚-25˚C.

8.3 Preparation of Standard Diluent Buffer

Add the contents of the vial (10x concentrate) to 225 ml of distilled water before use.
This solution can be stored at 2-8℃ for up to 1 week.

8.4 Preparation of Standard

Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 3000 pg/ml of TRAIL Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 3000 to 93.75 pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200 µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 3000 pg/ml.
- Add 100 µl of standard diluent to the remaining standard wells B1 and B2 to F1 and F2.

- Transfer 100 µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.

- Continue this 1:1 dilution using 100 µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 3000 pg/ml to 93.75 pg/ml.

- Discard 100 µl from the final wells of the standard curve (F1 and F2).

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred into the relevant wells.

8.5 Preparation of Controls

The supplied Controls must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution at the concentration stated on the vial.
Do not store after use.

8.5 Preparation of Biotinylated anti-TRAIL

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-TRAIL with the biotinylated antibody diluent in an appropriate clean glass vial. Use volumes appropriate to the number of required wells. Please see example volumes below:

Number of wells required	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

8.6 Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5 µl vial with 0.5 ml of HRP diluent **immediately before use.** Do not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of wells required	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (µl)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

9. METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.
Prepare all reagents as shown in section 8.
Note: final preparation of Biotinylated Secondary Antibody (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

Assay Step		Details
1.	Addition	**Prepare standard curve** as shown in section 8.4 above.
2.	Addition	Add 100 µl of each **Sample, Control and zero (Standard Diluent)** in duplicate to appropriate number of wells.
3.	Addition	Add 50 µl of diluted **Biotinylated anti-TRAIL** to all wells.
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **3 hours.**
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.3 ml of **1x Washing Solution** into each well. c) Aspirate the contents of each well. d) Repeat step b and c another two times.
6.	Addition	Add 100 µl of **Streptavidin-HRP** solution into all wells.
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **30 minutes.**
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100 µl of ready-to-use **TMB Substrate Solution** solution into all wells.
12.	Incubation	Incubate in the dark for **12=15 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
13.	Addition	Add 100 µl of **H_2SO_4 Stop Reagent** into all wells.
Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wave length and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).		

***** *Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore, the color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

10. DATA ANALYSIS

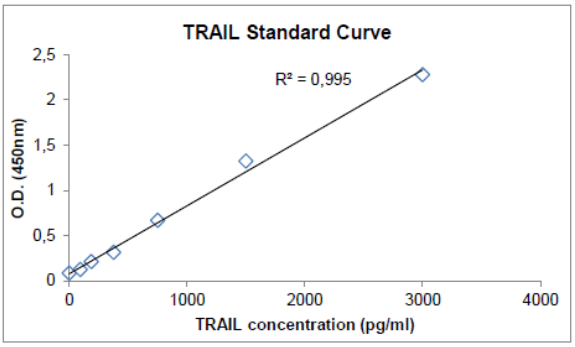
Calculate the average absorbance values for each set of duplicate standards and samples**.** Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding human TRAIL standard concentration on the horizontal axis.

The amount of TRAIL in each sample is determined by extrapolating OD values against TRAIL standard concentrations using the standard curve**.**

Sample TRAIL Standard Curve

Standard	TRAIL Conc (pg/ml)	OD (450nm) mean	CV (%)
1	3000	2.279	9.1
2	1500	1.322	5.4
3	750	0.669	1.2
4	375	0.313	1.5
5	187.5	0.210	1.5
6	93.75	0.125	5.7
Zero	0	0.083	-



Note: Curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

11. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle, and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

12. PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

The minimum detectable dose of TRAIL using this assay is **<64 pg/ml.** This has been determined by adding 2 standard deviations to the mean optical density obtained when the zero standard was assayed in 6 independent experiments.

12.2 Precision

Intra-Assay

Sample	n	Mean (pg/mL)	SD	CV%
A	40	1500	82.9	5.5
B	33	116.3	8.7	7.4

Inter-Assay

Sample	n	Mean (pg/mL)	SD	CV%
A	12	1619	62.8	3.8
B	12	162.8	13.1	8

12.3. Expected serum values

A panel of 50 human sera was tested for TRAIL. The detected level of TRAIL ranged from 430 to 1440 pg/ml with a mean of 973 +/- 263 pg/ml.

13. ASSAY SUMMARY

Total procedure length: 3 h 45 mn

↓

Add 100 µl of sample, controls and diluted standard and 50 µl Biotinylated anti-TRAIL.

↓

Incubate 3 hours at room temperature.

↓

Wash three times.

↓

Add 100 µl of Streptavidin-HRP.

↓

Incubate 30 min at room temperature.

↓

Wash three times.

↓

Add 100 µl of ready-to-use TMB.
Protect from light. Let the color develop for 12-15 min.

↓

Add 100 µl H_2SO_4

↓

Read Absorbance at 450 nm.

Cell Sciences ®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com