

Human TRAIL ELISA

Product Data Sheet

Cat. No.: RBMS2004R

For Research Use Only

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**»» This kit is manufactured by:
BioVendor – Laboratorní medicína, a.s.**

»» Use only the current version of Product Data Sheet enclosed with the kit!

1 INTENDED USE

The TRAIL ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human TRAIL in cell culture supernatants, human serum, plasma, or other body fluids. **The TRAIL ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

2 SUMMARY

TRAIL (TNF-related-apoptosis-inducing-ligand) was found to be a member of the TNF family which mediates cell death in a wide variety of malignant cell lines and primary tumor cells. TRAIL induces two different signals, cell death mediated by caspases and gene induction mediated by NFkappaB (9).

The cytotoxic ligand TRAIL interacts with five receptors, only two of which (TRAIL-R1 and R2) (2) have a death domain (5, 7, 14, 15).

TRAIL, which is ubiquitous expressed (6), exhibits very complex signalling and tumor-selective induction of apoptosis (1).

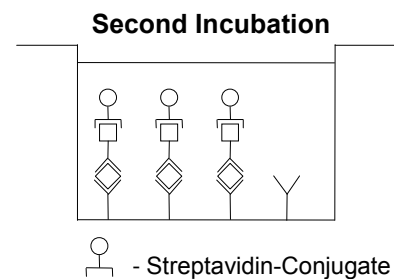
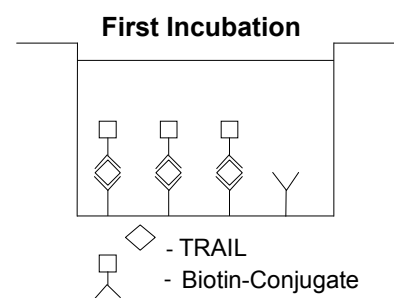
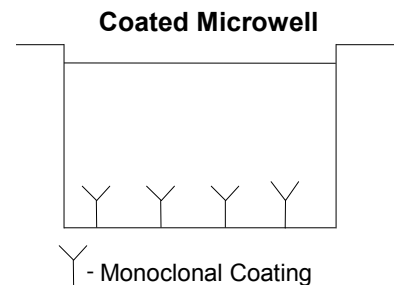
TRAIL is involved in lymphatic neoplastic disorders (6) and thyroid diseases (11), and the treatment of cancer (3, 4, 8, 12, 16) like melanoma (13).

3 PRINCIPLES OF THE TEST

An anti-TRAIL monoclonal coating antibody is adsorbed onto microwells.

TRAIL present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated monoclonal anti-TRAIL antibody is added and binds to TRAIL captured by the first antibody.

Following incubation unbound biotin conjugated anti-TRAIL is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-TRAIL. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.



4 REAGENTS PROVIDED

- 1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody to human TRAIL
- 1 vial (70 µl) **Biotin-Conjugate** anti-TRAIL monoclonal antibody
- 1 vial (150 µl) **Streptavidin-HRP**
- 2 vials **TRAIL Standard**, lyophilized, 2 ng/ml upon reconstitution
- 1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
- 1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10 % BSA)
- 1 vial (15 ml) **Substrate Solution**
- 1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)
- 1 vial (0.4 ml each) **Blue-Dye, Green-Dye, Red-Dye**
- 4 adhesive **Plate Covers**

Reagent Labels

5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on 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.

6 SPECIMEN COLLECTION

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.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive TRAIL. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 10 μ l to 1,000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ l adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis.

8 PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 PREPARATION OF REAGENTS

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with **Assay Buffer** (reagent B) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Preparation of TRAIL Standard

Reconstitute TRAIL **Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Mix gently to ensure complete solubilization.

E. Preparation of Streptavidin-HRP

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution in a clean plastic tube with **Assay Buffer** as needed according to the following table:

Number of Strips	Streptavidin- HRP (ml)	Assay Buffer (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

F. Addition of colour-giving reagents: **Blue-Dye, Green-Dye, Red-Dye**

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (**Blue-Dye, Green-Dye, Red-Dye**) can be added to the reagents according to the following guidelines:

- 1. Diluent:** Before sample dilution add the **Blue-Dye** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of **Blue-Dye**, proceed according to the instruction booklet.

5 ml Diluent	20 µl Blue-Dye
12 ml Diluent	48 µl Blue-Dye

- 2. Biotin-Conjugate:** Before dilution of the concentrated conjugate, add the **Green-Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of Biotin-conjugate.

3 ml Assay Buffer	30 µl Green-Dye
6 ml Assay Buffer	60 µl Green-Dye
12 ml Assay Buffer	120 µl Green-Dye

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the **Red-Dye** at a dilution of 1:250 (see table below) to the Dilution Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of **Red-Dye** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 μ l Red-Dye
12 ml Assay Buffer	48 μ l Red-Dye

10 TEST PROTOCOL

- a. Mix all reagents thoroughly without foaming before use.
- b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** to human TRAIL from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.
- c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

- d. Add 100 µl of **Assay Buffer** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (refer to preparation of reagents, 9.D.) **TRAIL Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of TRAIL standard dilutions ranging from 1000 to 15.6 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of TRAIL standard dilutions:

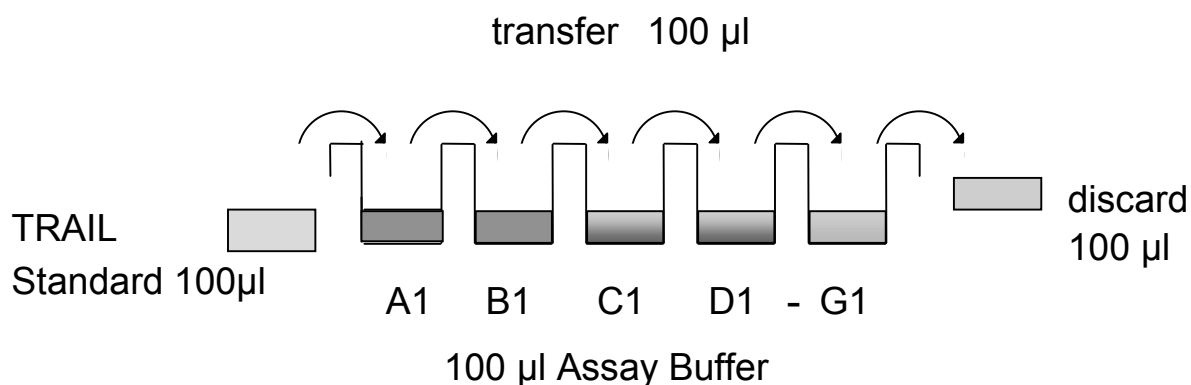


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1000 pg/ml)	Standard 1 (1000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (500 pg/ml)	Standard 2 (500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (250 pg/ml)	Standard 3 (250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (125 pg/ml)	Standard 4 (125 pg/ml)	Sample 4	Sample 4
E	Standard 5 (62.5 pg/ml)	Standard 5 (62.5 pg/ml)	Sample 5	Sample 5
F	Standard 6 (31.25 pg/ml)	Standard 6 (31.25 pg/ml)	Sample 6	Sample 6
G	Standard 7 (15.61 pg/ml)	Standard 7 (15.61 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

- e. Add 100 µl of **Assay Buffer** in duplicate to the blank wells.
- f. Add 50 µl of **Assay Buffer**, in duplicate, to the sample wells.
- g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.
- h. Prepare **Biotin-Conjugate** (refer to preparation of reagents, 9.C).
- i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.
- j. Cover with a **Plate Cover** and incubate at room temperature (**18° to 25°C**) for 2 hours, if available on a microplate shaker set at 100 rpm.
- k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.
- l. Prepare **Streptavidin-HRP** (refer to preparation of reagents 9.E).
- m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.
- n. Cover with a **Plate Cover** and incubate at room temperature (**18° to 25°C**) for 1 hour, if available on a microplate shaker set at 100 rpm.
- o. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.
- p. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

- q. Incubate the microwell strips at room temperature (**18° to 25°C**) for about **20 minutes**. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point r. of this protocol) before positive wells are no longer properly recordable.
It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.
- r. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.
- s. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the TRAIL standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the TRAIL concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating TRAIL for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding TRAIL concentration.

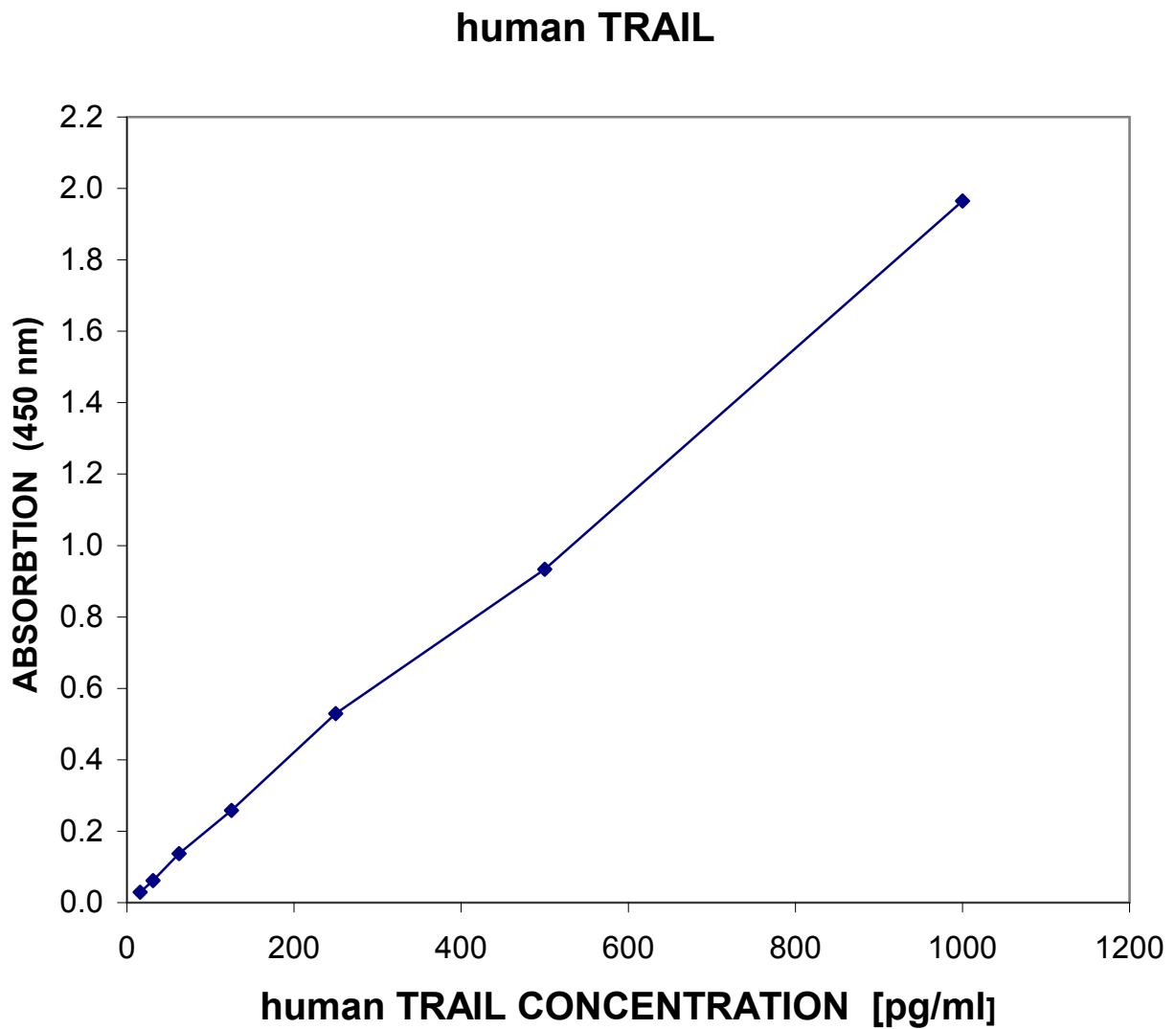
For samples which have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x 2)

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low TRAIL levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual TRAIL level.

It is suggested that each testing facility establishes a control sample of known TRAIL concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for TRAIL ELISA. TRAIL was diluted in serial two-fold steps in Assay Buffer, symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the TRAIL ELISA

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	TRAIL Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	CV %
1	1000	2.019	2.013	0.4
	1000	2.007		
2	500	0.978	0.982	0.6
	500	0.986		
3	250	0.557	0.578	0.1
	250	0.578		
4	125	0.293	0.307	6.2
	125	0.320		
5	62.5	0.185	0.186	0.4
	62.5	0.186		
6	31.25	0.116	0.111	7.0
	31.25	0.105		
7	15.6	0.082	0.078	7.3
	15.6	0.074		
Blank	0	0.049	0.049	
	0	0.048		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect enzymatic activity and thus colour intensity. Values measured are still valid.

12 LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- 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 Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

13 PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of TRAIL defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be less than 5.4 pg/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of TRAIL. Two standard curves were run on each plate. Data below show the mean TRAIL concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 6.5%.

Positive Sample	Experiment	TRAIL Concentration (pg/ml)	Coefficient of Variation (%)
1	1	867.0	3
	2	895.8	8
	3	861.8	9
2	1	229.2	8
	2	272.8	4
	3	204.6	5
3	1	156.4	8
	2	179.1	2
	3	133.2	9
4	1	396.1	6
	2	453.5	8
	3	410.9	5
5	1	259.2	6
	2	285.2	2
	3	228.5	4
6	1	677.3	8
	2	736.3	5
	3	625.6	3
7	1	515.8	10
	2	515.2	7
	3	497.2	7
8	1	70.3	8
	2	68.1	12
	3	70.1	10

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of TRAIL. Two standard curves were run on each plate. Data below show the mean TRAIL concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 7.7%.

Sample	TRAIL Concentration (pg/ml)	Coefficient of Variation (%)
1	874.9	2.1
2	235.5	14.7
3	156.2	14.7
4	420.2	7.1
5	257.6	11.0
6	679.7	8.2
7	509.4	2.1
8	69.5	1.7

C. Spiking Recovery

The spiking recovery was evaluated by spiking four levels of TRAIL into 4 different normal human sera. The amount of endogenous TRAIL in unspiked serum was subtracted from the spike values. Mean recovery was 77%.

D. Dilution Linearity

Four serum samples with different levels of TRAIL were assayed at three serial two-fold dilutions with 4 replicates each. Recoveries ranged from 94.0 % to 119.5 % with an overall mean recovery of 102.8%.

Sample	Dilution	TRAIL Concentration (pg/ml)		
		Expected Value	Observed Value	% Recovery of Exp. Value
1	1:2	--	815.5	--
	1:4	407.8	404.6	99.2 %
	1:8	203.9	210.3	103.2 %
	1:16	101.9	117.5	115.2 %
2	1:2	--	478.5	--
	1:4	239.3	225.0	94.0 %
	1:8	119.6	112.5	94.0 %
	1:16	59.8	71.5	119.5 %
3	1:2	--	195.3	--
	1:4	97.6	93.8	96.1 %
	1:8	48.8	50.5	103.4 %
	1:16	24.4	24.5	100.5 %

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of serum samples were stored frozen at -20°C and thawed up to 5 times, and TRAIL levels determined. There was no significant loss of TRAIL immunoreactivity in up to 5 cycles of freezing and thawing

b. Storage Stability

Aliquots of serum samples were stored at -20°C, 2-8°C, room temperature (RT), and 37°C and the TRAIL level determined after 24 h. There was no significant loss of TRAIL immunoreactivity during above storage conditions.

F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a serum. There was no detectable cross reactivity.

G. Expected Serum Values

A panel of 40 sera was tested for TRAIL. The detected TRAIL levels ranged between 30 and 136 pg/ml with a mean of 64pg/ml.

14 REFERENCES

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15 REAGENT PREPARATION SUMMARY

A. Wash Buffer Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

B. Assay Buffer	Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
	1 - 6	2.5	47.5
	1 - 12	5.0	95.0

C. Biotin-Conjugate Make a 1:100 dilution according to the table.

	Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (ml)
	1 - 6	0.03	2.97
	1 - 12	0.06	5.94

D. Standard Add distilled water to each vial of lyophilized **TRAIL Standard** (volume is stated on the label) as needed.

E. Streptavidin-HRP Make a 1:100 dilution according to the table.

	Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (ml)
	1 - 6	0.06	5.94
	1 - 12	0.12	11.88

16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Assay Buffer**, in duplicate, to all standard wells
- Pipette 100 µl reconstituted **TRAIL Standard** into the first wells and create standard dilutions ranging from 1000 to 15.61 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl **Assay Buffer**, in duplicate, to the blank wells
- Add 50 µl **Assay Buffer** to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature (**18° to 25°C**) on microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (**18° to 25°C**) on a microplate shaker
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µl of **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 20 minutes at room temperature (**18°to 25°C**)
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

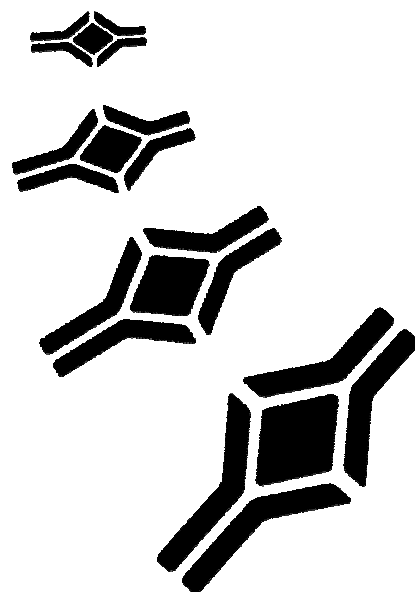
Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low TRAIL levels. Such samples require further dilution with Assay Buffer in order to precisely quantitate the actual TRAIL level.

NOTES



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