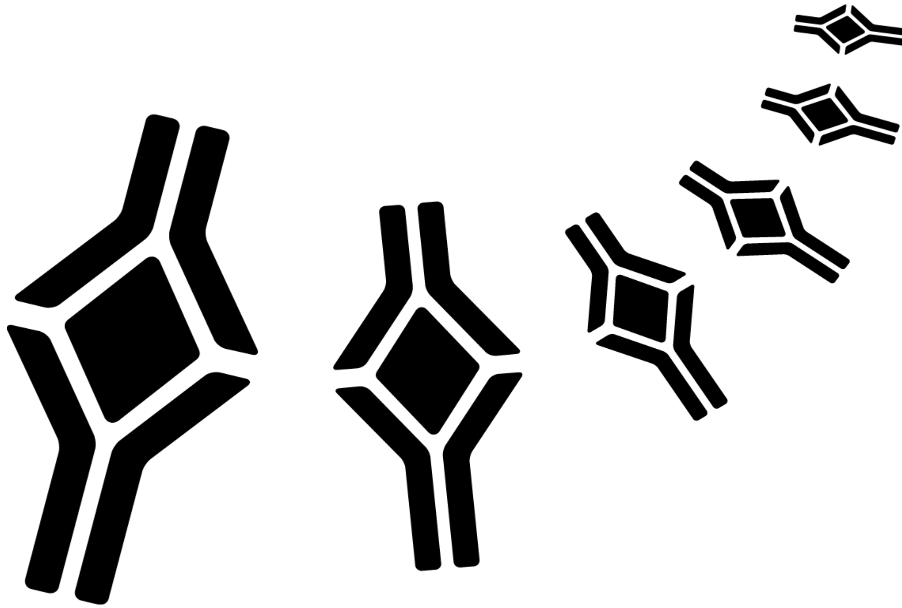

HUMAN TRAIL RECEPTOR 2 (CD262) ELISA

Product Data Sheet

Cat. No.: RGP023R

For Research Use Only

VERSION 51 131011 46

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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 Human Trail Receptor 2 (CD262) ELISA is to be used for research use only quantitative determination of TNF-related apoptosis-inducing -ligand Receptor 2 (Trail R2) in human serum, buffered solutions, cell culture medium or cell lysat. The assay will recognize both natural and recombinant human Trail R2. **This kit has been configured for research use only and is not to be used in diagnostic procedures.**

2. INTRODUCTION

Human Trail R2, also called DR5 (Death Receptor 5), CD262, TRICK 2 or Apo2 is a TNF receptor, which is a receptor for Trail (APO2 ligand). Trail R2 is involved in apoptosis. In the Trail receptor family, Trail R2, but also Trail R1 (DR4) transducer an apoptosis signal, whereas Trail R3 (DcR1) and Trail R4 (DcR2) antagonize TRAIL-induced apoptosis. Binding of trimeric Trail to Trail R1 induces apoptosis by oligomerization (likely to be trimerization) of the receptor. Trail R2 cDNA encodes a 440 amino acid residue precursor protein containing extracellular cysteine-rich domains, a transmembrane domain and a cytoplasmic death domain. Trail R2 shares 55% of its amino acid sequence with Trail R1.Like DR4, DR5 transcript is widely expressed in normal tissues and in many types of tumor cells.

3. PRINCIPLE OF THE METHOD

The Trail R2 Kit is a solid phase sandwich Enzyme Linked-Immuno- Sorbent Assay (ELISA). A monoclonal antibody specific for Trail R2 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Trail R2 concentrations and unknowns are pipetted into these wells.
During the first incubation, the Trail R2 antigen is added to wells. After washing, a biotinylated monoclonal antibody specific for Trail R2 is incubated. Then the enzyme (streptavidin-horse radish peroxydase) is added. After incubation and washing to remove all unbound enzyme, a substrate solution which actes on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of Trail R2 present in the samples.

4. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	**COLOUR CODE**	**Quantity**	**State**
Antibody Coated Microtiter Strips		96 wells	Ready to use
Plastic cover		2	
Standard: 1000 pg/ml	Yellow	2 vials	Reconstitute with the volume of standard diluent indicated on the vial. (See Reagents Preparation)
Standard Diluent buffer	Black	1 vial (25 ml)	10X concentrate. Dilute in distilled Water.
Biotinylated anti-Trail R2	Red	1 vial (0.4 ml)	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	Red	1 vial (7,5 ml)	Ready to use
Streptavidin-HRP		2 vials (5 µl)	0.5 ml of HRP-Diluent before further dilutions
HRP Diluent	Red	1 vial (23 ml)	Ready to use
Washing Buffer	White	1 vial (10 ml)	200X concentrated. Dilute in distilled Water
Chromogen TMB :		1 vial (11 ml)	Ready to use
H_2SO_4: Stop Reagent	Black	1 vial (11 ml)	Ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water.
- Pipettes : 10 µl, 50 µl, 100 µl, 200 µl and 1000 µl.
- Vortex mixer and magnetic stirrer.

6. SAFETY

- For research use only.
- The human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, 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.
- 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.

7. PROCEDURAL NOTES/LAB. QUALITY CONTROL

1. 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.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different lots.
5. Do not use reagents beyond the expiration date of the kit.
6. 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.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. 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.
10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
11. If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances rapidly after completion of the assay.
12. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
13. Respect incubation times described in the assay procedure.

8. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants - Remove particulates and aggregates by spinning at approximately 1000 g for 10 min.
Cell lysats - After spinning at approximately 400 g for 5 min, remove the supernatant and wash once again with PBS. Suspend cells in a cold lysis buffer. After 30 min of incubation, carefully remove the supernatant after spin at 10000 g for 10 min at 4°C. Store at -70°C.
Serum – Avoid any inintentional stimulation of the cells by the procedure. Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clothing. For that, after clothing, centrifuge at approximately 1000 g for 10 min and remove serum.
Storage - If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
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 assaying.

9. PREPARATION OF REAGENTS

9.1 Standard buffer diluent10X concentrate

Dilute 10 times with distilled water before use.

9.2 Standards

Standard have to be reconstituted with the volume of standard buffer diluent indicated on the vial. This reconstitution produces a stock solution of 1000 pg/ml Trail R2. Allow standard to stand for 5 minutes with gentle swirling prior to making dilutions. Serial dilutions of standard must be made before each assay and cannot be stored.

9.3 Sample

Dilution (½) of each sample with standard diluent is recommended (See Assay method e) and f)).

9.4 Dilution of biotinylated anti- Trail R2

Preparation immediately before use is recommended. Dilute the biotinylatedthe biotinylated anti Trail R2 with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

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

9.5 Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 µl vial of Streptavidin-HRP. DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS.

Dilute immediately before use. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

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

8.5 Washing Buffer 200X concentrate

Dilute 200 times in distilled water.

10. ASSAY METHOD

a) Before use, mix all reagents thoroughly without making foam.

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, and blank and optional control samples should be assayed **in duplicate**. Remove sufficient microwell strips from the pouch.

c) Add 100 µl of appropriateof standard diluent (see preparation of reagents) to standard wells B1, B2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells BB1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of TRAIL R2 standard dilutions ranging from 1000 to 31.25 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2). Alternatively these dilutions can be done in separate tube and diluted standard pipetted directly into wells.

d) Add 100 µl of standard diluent to the blank wells (G1-G2).

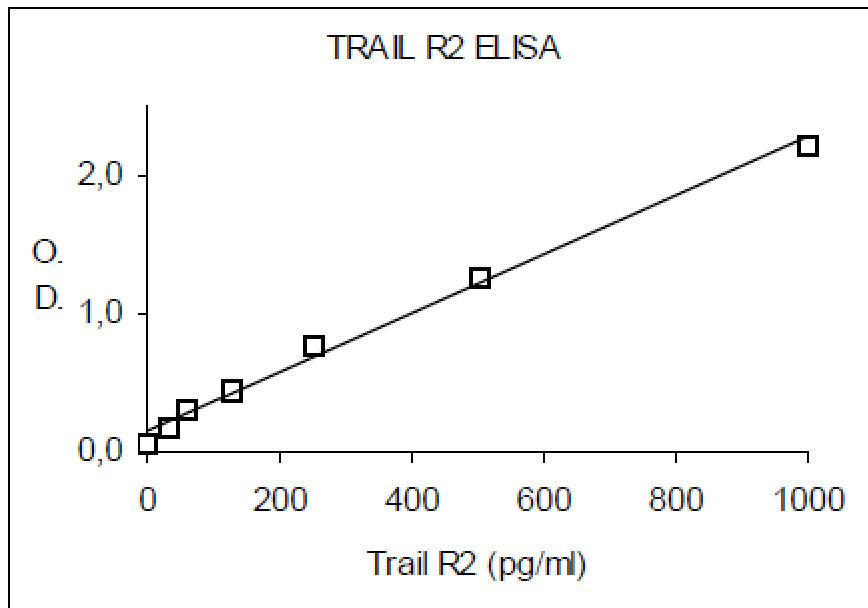
e) Add 50 µl of standard diluent to sample wells.
f) Add 50 µl of sample to sample wells. Mix the contents by repeated aspirations and ejections.
g) Add 100 µl of control to the control wells (H1; H2).
h) Cover with a plate cover and incubate for 1 hour at room temperature (18°C - 25°C).
i) Remove the cover and wash the plate as follows:
   1) aspirate the liquid from each well;
   2) dispense 0.3 ml of washing solution into each well;
   3) aspirate again the content of each well;
   4) Repeat steps 2) and 3) two times.
j) Preparation of biotinylated anti- TRAIL R2: (see preparation of reagents).
k) Add 50 µl of diluted biotinylated anti- TRAIL R2 to all wells.
l) Cover and incubate 1 hour at room temperature.
m) Wash as described in point h)
n) Prepare HRP solution just before use: (see preparation of reagents).
o) Dispense 100 µl of HRP solution into all wells, including the blank wells. Put back the cover.
p) Incubate the microwell strips at room temperature for 30 minutes.
q) Remove plate cover and empty wells. Wash microwell strips according to point h). Proceed immediately to the next step.
r) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 15-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
s)Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable.
t) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H_2SO_4: stop reagent into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read immediately after the addition of H_2SO_4: stop reagent.
u) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

11. SUGGESTED PLATE SCHEME

	Standard Concentrations pg/mL		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	1000	1000										
B	500	500										
C	250	250										
D	125	125										
E	62,5	62,5										
F	31,25	31,25										
G	Blank	Blank										
H												

12. DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding TRAIL R2 standard concentration on the horizontal axis. The amount of Trail R2 in each sample is determined by extrapolating OD values to TRAIL R2 concentrations using the standard curve.



Typical TRAIL R2 standard curve ranging from 1000 to 31.25 pg/mL

13. LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 1000 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples (> 1000 pg/ml) have to be tested with some dilutions with standard diluent or with your own sample buffer. Generally speaking, dilution (½) of each sample is recommended During analysis, multiply results by the appropriate dilution factor.
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been investigated. The rate of degradation of native TRAIL R2 in various matrices has not been investigated.

14. PERFORMANCES AND CHARACTERISTICS

14.1 Sensitivity

The minimum detectable dose of TRAIL R2 is less than 6 pg/ml.
This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times.

14.2 Expected values

Trail R2 isn't detected in healthy donor sera (64 sera).
Some cell lysats are Trail R2 positive like HeLa lysat. Concentrated PBMC supernatants are Trail R2 positive after stimulation by PMAionomicyne.

14.3 Precision

		Intra-Assay					Inter-Assay		
Sample	**n**	**Mean (pg/mL)**	**SD**	**CV%**	**Sample**	**n**	**Mean (pg/mL)**	**SD**	**CV%**
A	8	483	7	1,39	A	9	679	45	6,58
B	8	117	4	3,23	B	9	186	14	7,44

14.4 Dilution Linearity

A human serum pool containing 1000 pg/ml of measured Trail R2 was serially diluted in standard buffer diluent over the range of the assay.
Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99.

14.5 Recovery

Recovery of TRAIL R2 added to pooled normal serum is 106% (83% to 124%) for TRAIL R2 concentration ranging from 1000 to 250 pg/ml.

14.6 Specificity

No cross reactivity was observed with TRAIL, CD117, IL-6R, IL-2R, CD116, TRAIL R1, TRAIL R3, TRAIL R4, CD178 and Granzyme B.

15. REFERENCES

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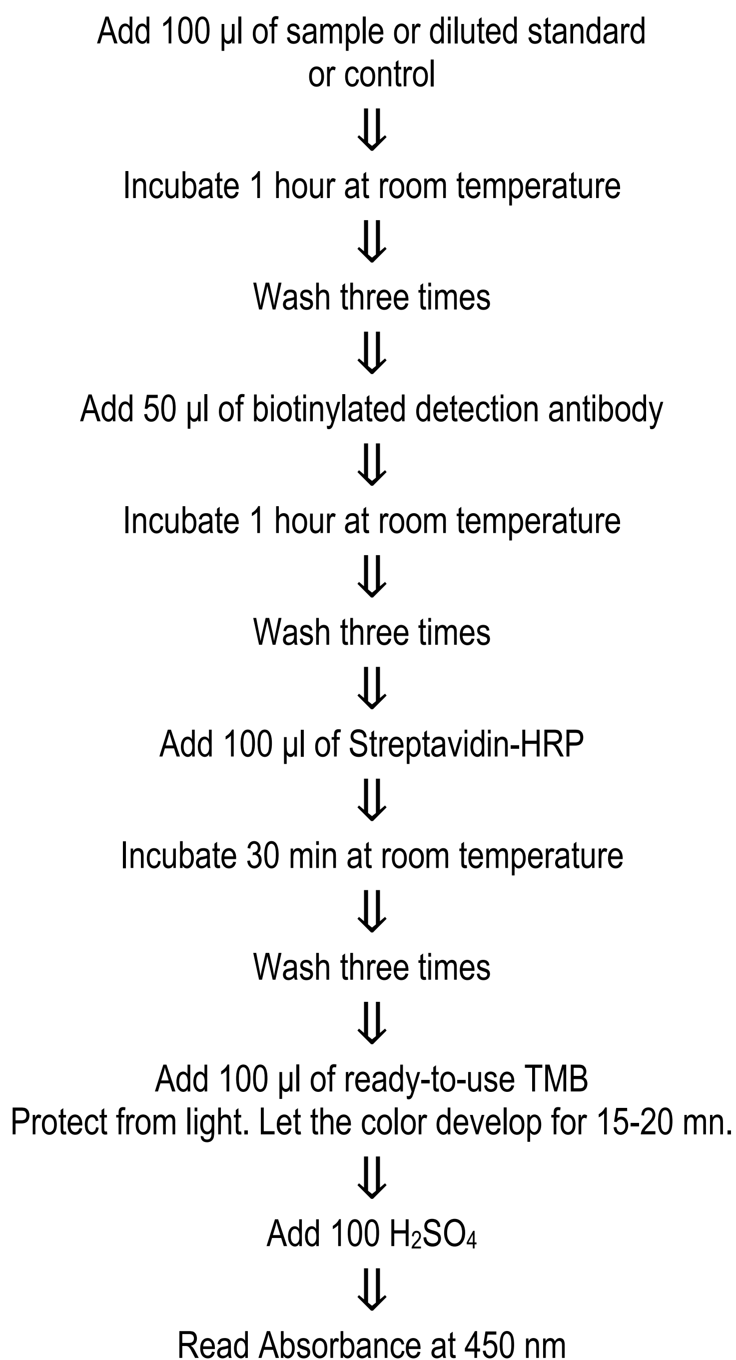
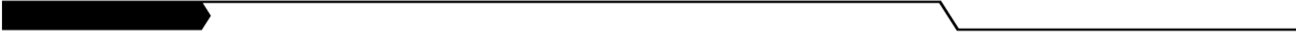
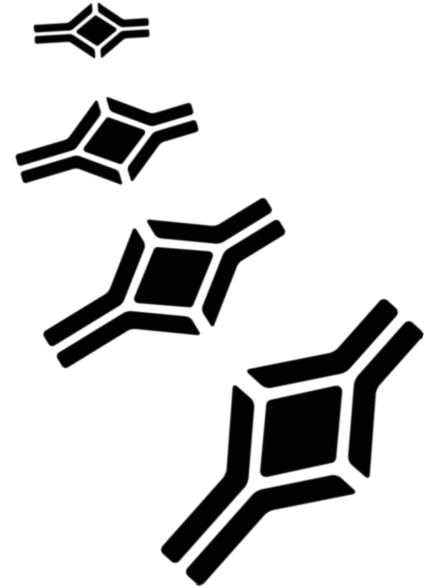
16. ASSAY PROCEDURE SUMMARY

Total procedure length 2 h 45 mn







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