Human CD262 / TRAIL-R2 ELISA Kit

Enzyme Immunoassay for the quantification of Human CD262 / TRAIL-R2 in Serum, plasma and cell culture supernatants

Catalog number: ARG81332

Package: 96 wells

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INTRODUCTION

The protein encoded by this gene is a member of the TNF-receptor superfamily, and contains an intracellular death domain. This receptor can be activated by tumor necrosis factor-related apoptosis inducing ligand (TNFSF10/TRAIL/APO-2L), and transduces an apoptosis signal. Studies with FADD-deficient mice suggested that FADD, a death domain containing adaptor protein, is required for the apoptosis mediated by this protein. Two transcript variants encoding different isoforms and one non-coding transcript have been found for this gene. [provided by RefSeq, Mar 2009]

Receptor for the cytotoxic ligand TNFSF10/TRAIL. The adapter molecule FADD recruits caspase-8 to the activated receptor. The resulting death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation which initiates the subsequent cascade of caspases (aspartate-specific cysteine proteases) mediating apoptosis. Promotes the activation of NF-kappa-B. Essential for ER stress-induced apoptosis. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CD262 / TRAIL-R2 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CD262 / TRAIL-R2 present is bound by the immobilized antibody. A biotin-conjugated antibody specific for CD262 / TRAIL-R2 is then added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-

enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CD262 / TRAIL-R2 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of CD262 / TRAIL-R2 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 vials (1000 pg/ml)	4°C
10X Standard/Sample diluent	1 X 15 ml	4°C
Antibody conjugate concentrate	1 vial (400 µl)	4°C
Antibody diluent buffer	7 ml (Ready to use)	4°C
HRP-Streptavidin concentrate	2 vials (5 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
200X Wash buffer	10 ml	4°C
TMB substrate	11 ml (Ready to use)	4°C (Protect from light)
STOP solution	11 ml (Ready to use)	4°C
Plate sealer	2 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex mixer
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 200X Wash buffer or Standard/Sample diluent concentrate, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards and samples be assayed in duplicates.
- We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation (1000 X g, 10 min) and aliquot & store samples at -70°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for few minutes before centrifugation for 10 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -70°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA, citrate or heparin as an anticoagulant. Centrifuge (1000 x g) for 30 minutes within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-70°C. Avoid repeated freeze-thaw cycles.

Note:

- It is recommended that thaw the samples at room temperature and make sure that sample is completely thawed and homogeneous before use. Do not thaw by heating at 37°C or 56°C.
- Avoid using haemolysed or lipemic samples.
- If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

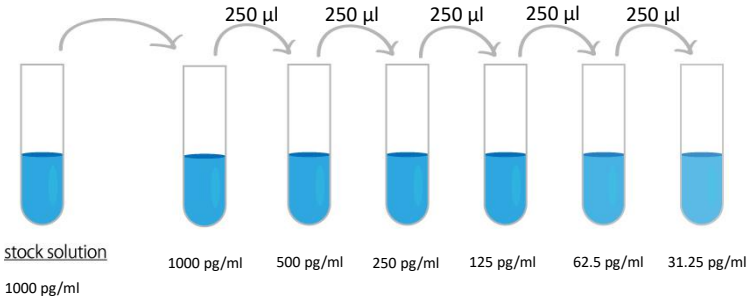
REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer (e.g. 10 ml 200X Wash buffer + 1990 ml distilled water). Store the 1X wash buffer at 2-8°C for up to 1 week.
- 1X Antibody conjugate: Prepare immediately before use, dilute 27.5X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution (e.g. for 16 wells, add 40 µl Antibody conjugate concentrate into 1060 µl Antibody diluent buffer). Use 1X Antibody conjugate immediately and do not store.
- HRP-Streptavidin working solution: Prepare immediately before use, it is recommended to centrifuge the vial for a few seconds to spin down all the volume at the bottom. Add 0.5 ml of HRP-Streptavidin diluent buffer in the vial that contains 5 µl of HRP-Streptavidin concentrate solution, mix completely to get the 1st diluted HRP-Streptavidin Solution. Then further dilute the solution 1:66.67 with HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin working Solution. (e.g. for 16 wells, add 30 µl of 1st diluted HRP-Streptavidin Solution into 2ml of HRP-Streptavidin diluent buffer). Use 1X HRP-Streptavidin working Solution immediately and do not store.
- 1X Standard/Sample Diluent Buffer: Dilute 10X Standard/Sample Diluent Buffer into distilled water to yield 1X Standard/Sample Diluent Buffer (e.g. 15 ml Standard/Sample Diluent Buffer + 135 ml distilled water). Store the 1X Standard/Sample Diluent Buffer at 2-8°C for up to 1 week.

- **Sample**: Human serum or plasma samples have to be diluted 1:2 in 1X Standard/Sample Diluent Buffer before assay. If the initial assay found samples contain CD262 / TRAIL-R2 higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: immediately before use, reconstitute the standard with **1 ml** of 1x Standard/Sample diluent to yield a stock concentration of **1000 pg/ml**. Mix the reconstituted standard gently by inversion only, and make sure the standard is dissolved completely before making serial dilutions. The 1x Standard/Sample diluent serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with 250 µl 1x Standard/Sample diluent as according to the suggested concentration below: **1000 pg/ml**, **500 pg/ml**, **250 pg/ml**, **125 pg/ml**, **62.5 pg/ml**, **31.25 pg/ml**.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (18 - 25°C) before use, each vial should be mixed thoroughly without foaming prior to use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples and zero standard (Standard/Sample diluent)** into wells. Cover wells and incubate for **1 hour at room temperature.**
3. Aspirate each well and wash, repeating the process two times for a total **three washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **50 µl** of **1X Antibody conjugate** into each well. Cover wells and incubate for **1 hour at room temperature**.
5. Aspirate each well and **wash** as step 3.
6. Add **100 µl** of **1X HRP-Streptavidin working solution** to each well. Cover wells and incubate for **30 minutes at room temperature**.
7. Aspirate each well and **wash** as step 3.
8. Add **100 µl** of **TMB substrate** reagent to each well. Incubate for **10-20 minutes at room temperature** in dark.
9. Add **100 µl** of **Stop Solution** to each well. The color of the solution should

change from blue to yellow. Gently tap the plate to ensure thorough mixing.

10. Read the OD with a microplate reader at **450 nm immediately**. (optional: read at 610-650 nm as the reference wave length) It is recommended to read the absorbance within 30 minutes after adding the stop solution.

    Note: 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.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

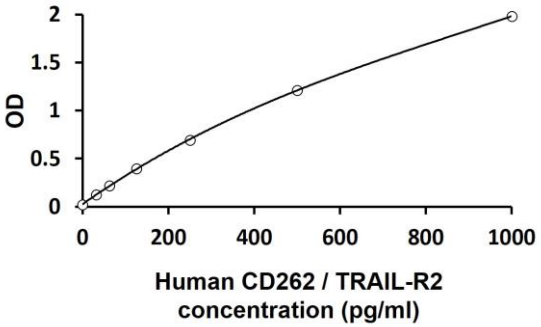
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

7. Expected serum/plasma values in healthy donor: It is not detected in sample from healthy donor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human CD262 / TRAIL-R2 ranged from 31.25- 1000 pg/ml. The mean MDD was 6 pg/ml.

Specificity

This assay recognizes natural soluble Human CD262 / TRAIL-R2. No significant cross-reactivity or interference with the factors below was observed:

TRAIL, CD117, IL6R, IL2R, CD116, CD261/TRAIL-R1, CD263/TRAIL-R3, CD264/TRAIL-R4, CD178 and Granzyme B

Intra-assay and Inter-assay precision

Intra-assay: 6.5%.

Inter-assay: 7.4%

Recovery range:

83- 124%