HUMAN THROMBOSPONDIN-2 (TSP-2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TSP-2 CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA Name	Human Thrombospondin-2 (TSP-2) ELISA
Catalog No.	SK00336-01
Formulation	96 T
Standard range	156-10000 pg/mL
Sensitivity	78 pg/mL
Sample require	30 µl
Dilution Factor	*10 (Optimal dilutions should be determined by each laboratory for each application.)*
Sample Type	Serum, EDTA Plasma
Specificity	Human TSP-2
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human Thrombospondin-2 Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human Thrombospondin-2 in serum and plasma. It contains recombinant Human Thrombospondin-2 and antibodies raised against this protein. It has been shown to accurately quantitie recombinant Human Thrombospondin-2. Results obtained with naturally occurring Thrombospondin-2 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural Human Thrombospondin-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Thrombospondin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Thrombospondin-2 present is bound by the immobilized antibody. After washing away any unbound substances, a Biotinylated polyclonal antibody specific for Thrombospondin-2 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Thrombospondin-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Code	Quantity
Thrombospondin-2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal IgG against Thrombospondin-2.	336-01-01	1 plate
THROMBOSPONDIN-2 Standard – 20 ng/vial of recombinant Human Thrombospondin-2 in a buffered protein base with preservatives; lyophilized.	336-01-02	1 vial
Detection Antibody– 1.2 mL / vial, 10-fold concentrated of a purified polyclonal IgG Biotinylated against THROMBOSPONDIN -2 with preservatives; lyophilized.	336-01-03	1 vial
Positive Control – one vial of recombinant Thrombospondin-2 , lyophilized	336-01-04	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer- 60mL/vial of buffered protein based solution with preservatives	DB06	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution-13 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution（0.5M HCL）, 13 ml /vial of 0.5M HCL	S-STOP	1 vial

Plate Covers – Plate sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: May be stored for up to 1 month at 2 - 8°C.
Standard : Reconstituted standard should be stored for up to two weeks at -70° C. *Diluted standard working solution and Positive Control should be prepared and used immediately*.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 8 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. 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.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

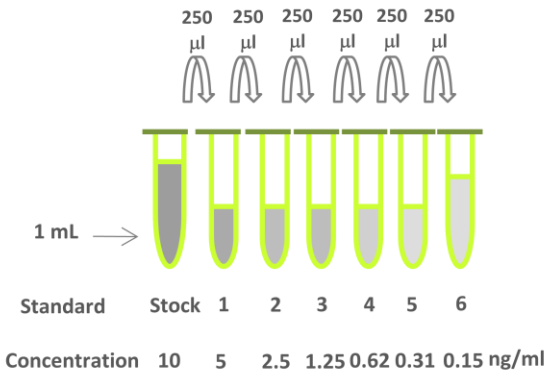
SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 30 µL sample + 270µ L Dilution Buffer. **Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
Thrombospondin-2 Standard - Refer to vial label for reconstitution volume. Reconstitute the **Thrombospondin-2** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

Standard	Standard	Reagent Diluent	Concentration
stock	powder	1 ml	10000pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	312.5 pg/ml
# 6	250µl of 5	250µl	156 pg/ml



Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of the appropriate Dilution Buffer into the 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11. 88 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*.

ASSAY PROCEDURE

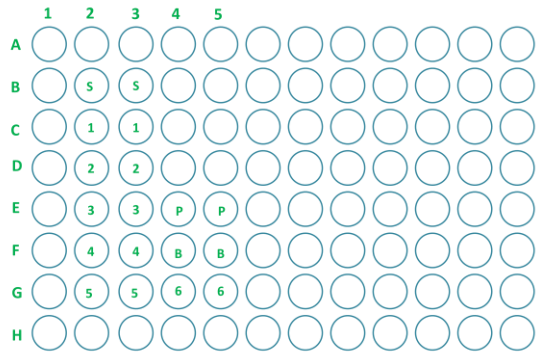
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of Standard (from B2 to G3, G4 to G5), sample, or positive control per well. Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 20-30 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Thrombospondin-2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



Calculation of samples with a concentration exceeding that of standard 10 ng/ml may result in inaccurate, low human Thrombospondin-2 levels. Such samples require further external predilution according to expected human Thrombospondin-2 values with Dilution Buffer in order to precisely quantitate the actual human Thrombospondin-2 level.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0 expressed recombinant Human Thrombospondin-2/OSF-2.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Thrombospondin-2
Was 78 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human Thrombospondin-2. The factors listed below were prepared at 100 ng/mL in Dilution Buffer, and assayed for cross reactivity.

Protein Name	Cross-reactivity
Human Thrombospondin-2	100%
Human Thrombospondin-1	0
Human Thrombospondin-4	0
Human CD36	0

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptatvin HRP conjugate to each well. Incubate 1 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate to each well. Incubate 20-30 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min