AssayMax Human Tissue Factor (TF) ELISA Kit

Catalog Number ET1002-1

Introduction

The transmembrane protein Tissue factor (TF) is the physiologic trigger of coagulation in normal hemostasis. TF binds and allosterically activates factor VII. The TF-VIIa complex cleaves factor IX and X, leading to thrombin generation (1). Inducible expression of TF in a variety of pathological conditions, including gram-negative sepsis and acute coronary syndromes, is associated with life-threatening thrombosis (2, 3). In sepsis, TF expression within the vasculature leads to disseminated intravascular coagulation (4). TF also plays important roles in vasculogenesis, metastasis, and tumor-associated angiogenesis (5, 6, 7).

Principal of the Assay

The AssayMax Human Tissue Factor (TF) ELISA kit is designed for detection of human TF in plasma, tissue, and cell culture lysate. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TF in 4 hours. A murine monoclonal antibody specific for TF has been pre-coated onto a 96-well microplate. TF in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for TF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **TF Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against TF
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **TF Standard:** Human recombinant TF in a buffered protein base (800 pg, lyophilized)
- **Biotinylated TF Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against TF (80 µl)
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (120 µl)

- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml)
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml)
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel pipettes)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000x g for 10 minutes. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay immediately. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Lysates:** The cultured cells are lysed and solubilized with 15 mM octyl-β-D-glucopyranoside at 37^{0}C for 15 minutes. Collect fresh cell lysates. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -20^{0}C or below.
- **Tissue:** Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14,000x g for 20 min. Collect the supernatant and measure the protein concentration. Use undiluted samples or 1:2 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -20^{0}C or below.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **TF Standard:** Reconstitute the 800 pg of TF Standard with 2.0 ml of EIA Diluent to generate a 400 pg/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution twofold with equal volume of EIA Diluent to produce 200, 100, 50, 25, 12.5, and 6.25 pg/ml. EIA Diluent serves as the zero standard (0 pg/ml).

Standard Point	Dilution	[TF] (pg/ml)
P1	1 part TF Standard	400.00

P2	1 part P1 + 1 part EIA Diluent	200.00
P3	1 part P2 + 1 part EIA Diluent	100.00
P4	1 part P3 + 1 part EIA Diluent	50.00
P5	1 part P4 + 1 part EIA Diluent	25.00
P6	1 part P5 + 1 part EIA Diluent	12.50
P7	1 part P6 + 1 part EIA Diluent	6.25
P8	EIA Diluent	0.00

- **EIA Diluent Concentrate (10x)**: Dilute the EIA Diluent 1:10 with reagent grade water.
- **Biotinylated TF Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents. Hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated TF Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 20 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

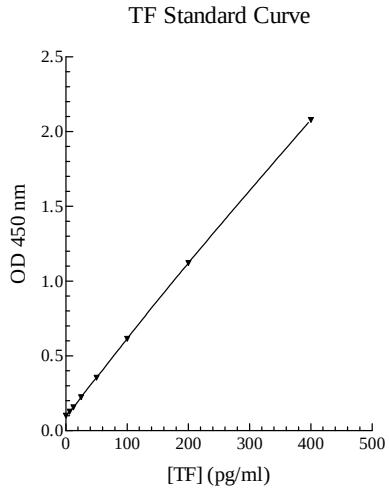
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter or linear curve.

- Determine the unknown sample concentration from the Standard Curve and multiply the plasma or tissue value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of TF is typically 6 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.9% and 7.0% respectively.
- This assay recognizes both natural and recombinant human TF apoprotein and TF/FVII complexes. No significant cross-reactivity or interference was observed.

References

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