



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, serum, tissue, and cell culture lysate. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TF in 4 hours. A polyclonal 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 polyclonal 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 (400 pg, lyophilized).
- **Biotinylated TF Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against TF (80 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).

- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C 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 μ l, 20-200 μ l, 200-1000 μ l 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 2000 x g for 10 minutes. Use undiluted samples or 1:4 diluted samples with MIX Diluent and assay immediately. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Dilute samples 1:4 into MIX Diluent and assay. The undiluted samples can be stored at -20⁰C 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 37⁰C for 15 minutes. Collect fresh cell lysates. Use undiluted samples or 1:2 diluted samples with MIX Diluent and assay. The undiluted samples can be stored at -20⁰C or below.
- **Tissue:** Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000x g for 20 min. Collect the supernatant and measure the protein concentration. Use undiluted samples or 1:2 diluted samples with MIX Diluent and assay. The undiluted samples can be stored at -20⁰C 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.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **TF Standard:** Reconstitute the 400 pg of TF Standard with 1 ml of MIX 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 MIX Diluent to produce 200, 100, 50, 25, 12.5 pg/ml. MIX Diluent serves as the zero standard (0 pg/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[TF] (pg/ml)
P1	1 part TF Standard	400.00
P2	1 part P1 + 1 part MIX Diluent	200.00
P3	1 part P2 + 1 part MIX Diluent	100.00
P4	1 part P3 + 1 part MIX Diluent	50.00
P5	1 part P4 + 1 part MIX Diluent	25.00
P6	1 part P5 + 1 part MIX Diluent	12.50
P7	MIX Diluent	0.00

- **Biotinylated TF Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

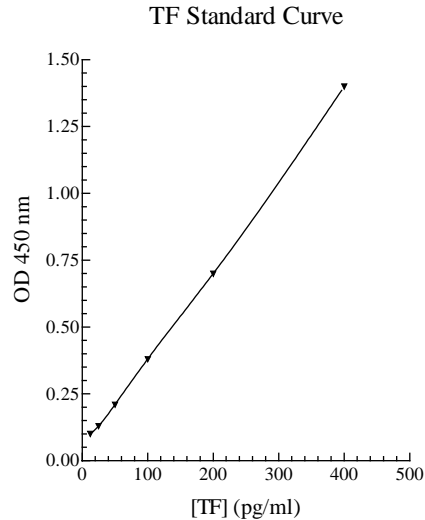
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal 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 with a sealing tape and incubate for two 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 two 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 10 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 using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the 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 10 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.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
No Dilution	97%	95%
1:2	100%	101%
1:4	110%	111%

Recovery

Standard Added Value	20 – 200 pg/ml
Recovery %	80-115 %
Average Recovery %	97.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 10 (suggest 1:2 dilution for plasma)
Monkey	< 10 (suggest 1:2 dilution for plasma)
Mouse	< 30 (suggest 1:4 dilution for plasma)
Rat	< 1
Swine	< 20 (suggest 1:2 dilution for plasma)

References

- (1) Ruf, W. and Edgington, T.S. (1994) *FASEB J.* 8:385
- (2) Fuster, V. *et al.* (1996) *Haemostasis* 26:269
- (3) Leatham, E. *et al.* (1995) *Br. Heart. J.* 73:10
- (4) Drake, T.A. *et al.* (1993) *Am. J. Pathol.* 142:1
- (5) Carmeliet, P. *et al.* (1996) *Nature* 383:73
- (6) Ruf, W. and Mueller, B.M. (1996) *Curr. Opin. Hematol.* 3:379
- (7) Zhang, Y. *et al.* (1994) *J. Clin. Invest.* 94:1320

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