



AssayMax Human Tissue Factor Pathway Inhibitor (TFPI) ELISA Kit

Catalog Number ET1005-1

Introduction

Tissue factor pathway inhibitor (TFPI) is an endogenous protease inhibitor that regulates the initiation of the extrinsic coagulation pathway by producing factor Xa-mediated feedback inhibition of the tissue factor/factor VIIa (TF/FVIIa) catalytic complex (1). TFPI has a negatively charged amino-terminus, three tandem Kunitz proteinase inhibitory domains, and a positively charged carboxy-terminus. The first Kunitz domain is the binding site for the TF/FVIIa complex and the second domain for factor Xa. The resultant quaternary complex of TFPI/FXa/TF/FVIIa lacks TF/FVIIa catalytic activity (2). The third Kunitz-type domain and the carboxy-terminus of TFPI mediate its binding to heparin and cell surfaces including the endothelium (3). TFPI is synthesized mainly by endothelial cells and present in three pools *in vivo*: 10% in platelets, in endothelium associated with endothelial glyco-saminoglycans, and in plasma circulating as free or lipoprotein associated forms (4). The plasma TFPI contains mostly 34 and 40 kDa forms and the concentration is approximately 50 to 100 ng/ml (5, 6). Measurement of TFPI could be important in thrombogenesis, atherosclerosis and heparinization studies. Higher plasma levels of TFPI were found in older individuals, pregnant women and patients with advanced cancer (7, 8, 9).

Principal of the Assay

The AssayMax Human TFPI ELISA kit is designed for detection of human TFPI in plasma and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TFPI in 4 hours. A murine antibody specific for TFPI has been pre-coated onto a 96-well microplate with removable strips. TFPI in standards and samples is sandwiched by the immobilized antibody and a polyclonal antibody specific for TFPI, which is recognized by a peroxidase conjugate. All unbound material is then washed away and a peroxidase conjugate 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

- **TFPI Microplate:** 96 well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against TFPI.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **TFPI Standard:** Human TFPI in a buffered protein base (25 ng, lyophilized).
- **TFPI Antibody (50x):** A 50-fold concentrated polyclonal antibody against human TFPI (80 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Peroxidase Conjugate (100x):** A 100-fold concentrate (80 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)
- 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 and assay. Dilute samples 1:20 with MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Dilute all reagents freshly 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.
- **Standard Curve:** Reconstitute the 25 ng of human TFPI Standard with 2.5 ml of MIX Diluent to generate a 10 ng/ml of stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the TFPI standard solution (10 ng/ml) twofold with equal volume of MIX Diluent to

produce 5, 2.5, 1.25 and 0.625 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[TFPI] (ng/ml)
P1	1 part Standard (10 ng/ml)	10.00
P2	1 part P1 + 1 part MIX Diluent	5.00
P3	1 part P2 + 1 part MIX Diluent	2.50
P4	1 part P3 + 1 part MIX Diluent	1.25
P5	1 part P4 + 1 part MIX Diluent	0.63
P7	MIX Diluent	0.00

- **TFPI Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **Peroxidase Conjugate (100x):** Spin down the Peroxidase Conjugate briefly and dilute the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

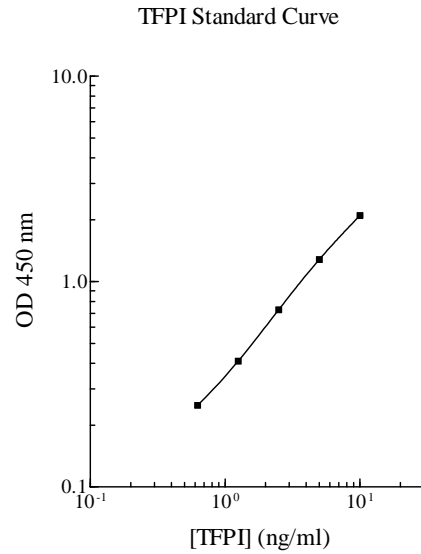
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Standard or sample per well, and immediately add 25 µl of Anti-TFPI Antibody to each well (on top of the standard or sample). Cover wells with a sealing tape and incubate for two hours at room temperature. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Peroxidase Conjugate to each well and incubate for one hour at room temperature. 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 to each well and incubate for about 10 minutes at room temperature or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles if there is any in the well with pipette tip.
- Add 50 µl of Stop Solution per 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 and subtract the mean value of zero standard readings.
- 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.



Precision, Sensitivity and Specificity

- The minimum detectable level of TFPI is typically < 0.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.0 % and 7.0% respectively.
- This kit measure total TFPI concentration.

Linearity

	Average Percentage of Expected Value
Sample Dilution	Plasma
1:10	95%
1:20	98%
1:40	101%

Plasma Value (Normal Plasma)

Average EDTA Plasma (n=30)	85 ng/ml
Average Citrated Plasma (n=30)	75 ng/ml
Average Heparin Plasma (n=30)	90 ng/ml

Recovery

Standard Added Value	1-10 ng/ml
Recovery %	89 - 111
Average Recovery %	100

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 30 (suggest 1:5 dilution for plasma/serum)
Mouse	None
Rat	None
Swine	< 5

References

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Version 8.2