TFPI (Human) ELISA kit

Catalog Number KA0507

96 assays

Version: 04

Intended for research use only

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Introduction

Background

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 is 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 glycosaminoglycans, 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 (7-9).

Principle of the Assay

TFPI (Human) ELISA kit is designed for detection of human TFPI in plasma, serum, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TFPI in less than 4 hours. A polyclonal 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 polyclonal antibody and biotinylated polyclonal antibody specific for TFPI, 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.

General Information

Materials Supplied

List of component

Component	Amount
Human TFPI Microplate: A polystyrene microplate coated with a polyclonal antibody against TFPI.	96 (8x12) well
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human TFPI Standard: Human TFPI in a buffered protein base, lyophilized.	15 ng
Biotinylated Human TFPI Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human TFPI.	140 µL
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 x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µL
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 Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution

Assay Protocol

Reagent Preparation

Freshly dilute all reagents and bring all reagents to room temperature before use.

- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.

- ✓ Standard Curve: Reconstitute the 15 ng of Human TFPI Standard with 1.5 mL of MIX Diluent to generate a stock solution of 10 ng/mL standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate points by serially diluting the standard (10 ng/mL) 1:2 using equal volume of MIX Diluent to produce 5, 2.5, 1.25, 0.625, 0.313 and 0,156 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at < -20°C and used within 30 days.

Standard Point	Dilution	[TFPI] (ng/mL)
P1	1 part Standard (10 ng/mL)	10.00
P2	1 part P1 + 1 part MIX Diluent	5.000
P3	1 part P2 + 1 part MIX Diluent	2.500
P4	1 part P3 + 1 part MIX Diluent	1.250
P5	1 part P4 + 1 part MIX Diluent	0.625
P6	1 part P5 + 1 part MIX Diluent	0.313
P7	1 part P6 + 1 part MIX Diluent	0.156
P8	MIX Diluent	0.000

- ✓ Biotinylated Human 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): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 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.

Sample Preparation

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes. Dilute samples 1:40 with MIX Diluent or within the range of 1:10 to 1:80 into MIX Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA and Heparin can also be used as an anticoagulant).

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. Dilute samples 1:40 into MIX Diluent or within the range of 1:10 to 1:80 into MIX Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes and assay. Store samples at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Human TFPI Standard or sample per well. Cover wells with a sealing tape, and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.Add 50 µL of Biotinylated Human TFPI Antibody to each well, and incubate for 1 hour.
5. Wash the microplate as described above.
6. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes at room temperature. Turn on the microplate reader and set up the program in advance.
7. Wash the microplate as described above.
8. Add 50 µL of Chromogen Substrate to each well and incubate for about 7 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.
9. Add 50 µL of Stop Solution per well. The color will change from blue to yellow.
10. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that someunstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings

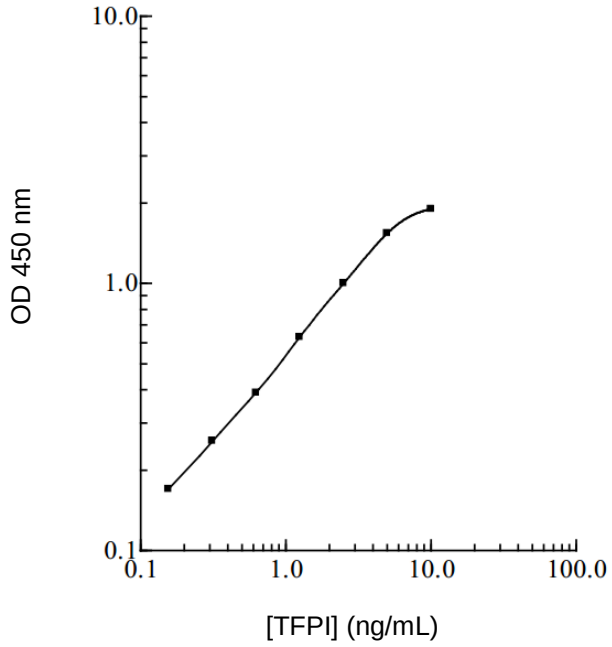
✓ Summary

1. Add 50 µL of Standard/Sample per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 7 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or 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 byregression 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.
- ✓ 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 TFPI is typically ~0.1 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8% and 7.1% respectively.
- ✓ This kit measures total TFPI concentration.

- ✓ Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:20	89%	91%
1:40	100%	98%
1:80	107%	104%

- ✓ Recovery

Standard Added Value	0.313 - 5.0 ng/mL
Recovery %	89 - 107 %
Average Recovery %	98 %

- ✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	<30%
Mouse	<5%
Rat	None
Swine	<10%
Rabbit	None
Human	100%

- ✓ Reference Values

Normal human TFPI plasma levels range from 50 to 100 ng/mL.

Resources

References

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Plate Layout

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