

Human TFPI Sandwich ELISA Kit Datasheet

For the quantitative detection of human TFPI concentrations in serum, plasma and cell culture supernatants.

General Information

| | |
|---------------------------|-----------------------------------|
| Catalogue Number | KE00202 |
| Product Name | Human TFPI Sandwich ELISA Kit |
| Species cross-reactivity | Human |
| Range (calibration Range) | 15.6-1000 pg/mL , 62.5-2000 pg/mL |
| Tested applications | Quantification ELISA |

Database Links

| | |
|-------------|--------|
| Entrez Gene | 7035 |
| SwissProt | P10646 |

Kit Components & Storage

| | | |
|--|-----------|---|
| Microplate - antibody coated 96-well microplate (8 well × 12 strips) | 1 plate | Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay. |
| Protein standard - 2000 pg/bottle; lyophilized* Reconstitution by Sample Diluent PT 3-eg | 2 bottles | |
| Protein standard - 1000 pg/bottle; lyophilized** Reconstitution by Sample Diluent PT 4 | 2 bottles | |
| Detection Antibody, biotinylated (100X) - 120 µL/vial | 1 vial | |
| Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial | 1 vial | |
| Sample Diluent PT 3-eg - 30 mL/bottle. For serum and plasma | 1 bottle | |
| Sample Diluent PT 4 - 30 mL/bottle. For cell culture supernatants | 1 bottle | |
| Detection Diluent - 30 mL/bottle | 1 bottle | |
| Wash Buffer Concentrate (20X) - 30 mL/bottle | 1 bottle | |
| Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle | 1 bottle | |
| Stop Solution - 12 mL/bottle | 1 bottle | |
| Plate Cover Seals | 3 pieces | |

NB: Do not use the kit after the expiration date.

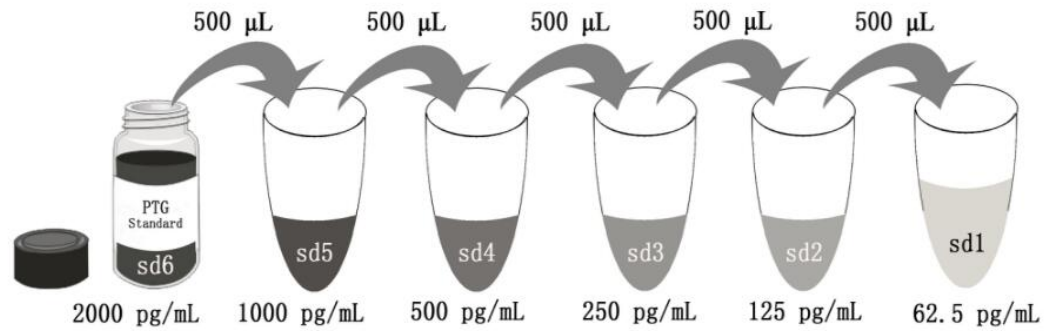
Sample Diluent PT 3-eg is for protein standard, serum and plasma.

Sample Diluent PT 4 is for protein standard and cell culture supernatants.

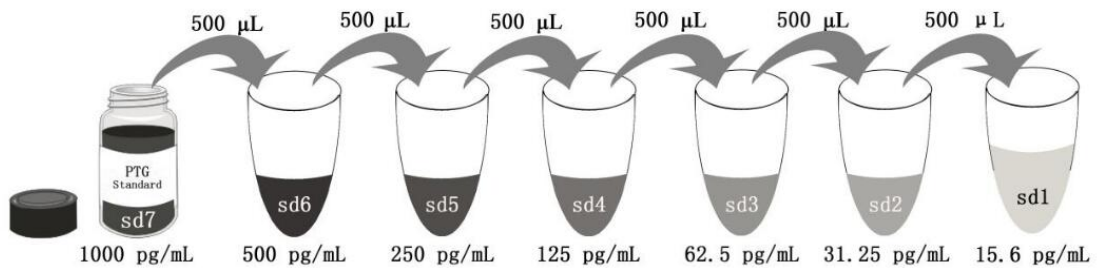
Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 3-eg in protein standard (2000 pg/bottle). This reconstitution gives a stock solution of 2000 pg/mL.

**Add 1 mL Sample Diluent PT 4 in protein standard (1000 pg/bottle). This reconstitution gives a stock solution of 1000 pg/mL.



| | | | | | | |
|--|--------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Add # μL of Standard diluted in the previous step | — | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
| # μL of Sample Diluent PT 3-eg | 1000 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
| | "sd7" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |



| | | | | | | | |
|--|--------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Add # μL of Standard diluted in the previous step | — | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
| # μL of Sample Diluent PT 4 | 1000 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

Product Description

KE00202 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TFPI ELISA kit is to be used to detect and quantify protein levels of endogenous TFPI. The assay recognizes human TFPI. An antibody specific for TFPI has been pre-coated onto the microwells. The TFPI protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for TFPI is added to detect the captured TFPI protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Tissue factor pathway inhibitor (TFPI) is a critical anticoagulant protein present in endothelium and platelets. TFPI is produced as two major isoforms in humans, TFPIa and TFPIb that result from alternative splicing. The TFPIb isoform localizes to the endothelium surface where it is a potent inhibitor of tissue factor-factor VIIa complexes that initiate blood coagulation. The TFPIa isoform is present in platelets. TFPI is a Kunitz-type serine protease inhibitor that exerts anticoagulant activity by blocking early procoagulant stimuli. TFPI can enhance anti-thrombotic treatment in sepsis, inflammatory diseases, and cardiovascular diseases.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:10 dilution is recommended for serum or plasma and cell culture supernatants.

Safety Notes

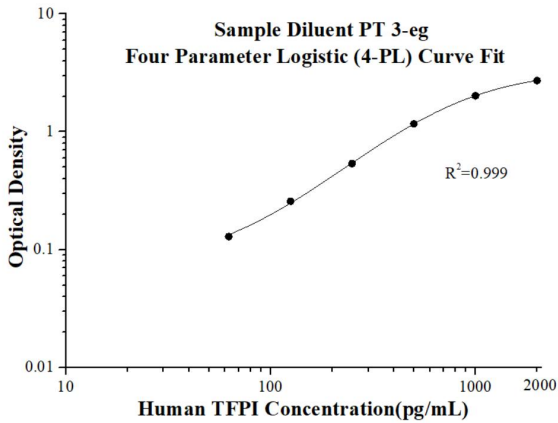
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

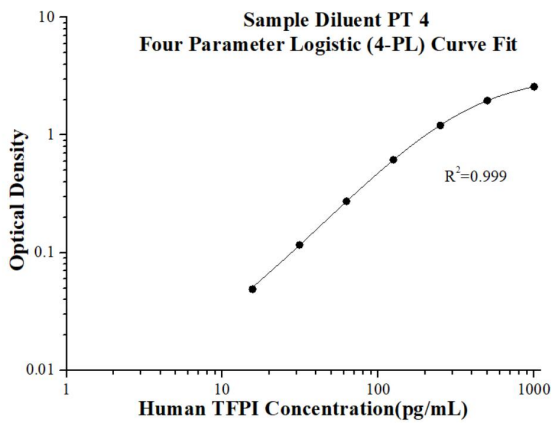
| Step | Reagent | Volume | Incubation | Wash | Notes |
|------|--|--------|------------|-------------|------------------------------|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

Example data

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



| (pg/mL) | O.D | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.044 0.037 | 0.040 | - |
| 62.5 | 0.171 0.168 | 0.170 | 0.130 |
| 125 | 0.298 0.298 | 0.298 | 0.258 |
| 250 | 0.583 0.573 | 0.578 | 0.538 |
| 500 | 1.209 1.209 | 1.209 | 1.169 |
| 1000 | 2.093 2.035 | 2.064 | 2.024 |
| 2000 | 2.775 2.753 | 2.764 | 2.724 |



| (pg/mL) | O.D | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.085 0.082 | 0.084 | - |
| 15.6 | 0.129 0.136 | 0.133 | 0.049 |
| 31.25 | 0.194 0.206 | 0.200 | 0.116 |
| 62.5 | 0.362 0.352 | 0.357 | 0.273 |
| 125 | 0.694 0.706 | 0.700 | 0.616 |
| 250 | 1.279 1.305 | 1.292 | 1.208 |
| 500 | 2.043 2.056 | 2.050 | 1.966 |
| 1000 | 2.630 2.689 | 2.660 | 2.576 |

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | | Inter-assay Precision | | | | |
|-----------------------|----|--------------|------|-----|-----------------------|----|--------------|------|-----|
| Sample | n | Mean (pg/mL) | SD | CV% | Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 23.3 | 2.2 | 9.4 | 1 | 24 | 22.9 | 1.5 | 6.6 |
| 2 | 20 | 75.7 | 5.4 | 7.1 | 2 | 24 | 66.8 | 5.6 | 8.5 |
| 3 | 20 | 312.9 | 15.6 | 5.0 | 3 | 24 | 314.2 | 18.1 | 5.8 |

Recovery

The recovery of TFPI spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---------------------------|------|----------------------|-----------|
| Human plasma | 1:40 | 113 | 104-122 |
| | 1:80 | 101 | 75-117 |
| Cell culture supernatants | 1:40 | 96 | 72-116 |
| | 1:80 | 82 | 72-120 |

Sample Values

Serum and plasma samples from healthy volunteers were evaluated for TFPI in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Range (pg/mL) | Mean (pg/mL) |
|---------------|-------------------|--------------|
| Serum (n=24) | 11,374.5-34,544.1 | 21,718.9 |
| Plasma (n=24) | 3,102.5-38,479.1 | 21,784.8 |

Cell Culture supernatants

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human TFPI, and measured 4.1 ng/mL.

HUVEC human umbilical vein endothelial cells were cultured in EGM for 3 days. An aliquot of the cell culture supernate was removed, assayed for human TFPI, and measured 12.7 ng/mL.

Sensitivity

The minimum detectable dose of TFPI is 7.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, Serum and cell culture supernatants samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

| Sample Type | | Range (%) | Average% of Expected |
|---------------------------|------|-----------|----------------------|
| Human plasma | 1:10 | - | 100 |
| | 1:20 | 90-95 | 92 |
| | 1:40 | 89-93 | 91 |
| | 1:80 | 84-101 | 92 |
| Human serum | 1:10 | - | 100 |
| | 1:20 | 91-102 | 97 |
| | 1:40 | 93-102 | 97 |
| | 1:80 | 96-101 | 98 |
| Cell culture supernatants | 1:10 | - | 100 |
| | 1:20 | 101-103 | 102 |
| | 1:40 | 95-99 | 97 |
| | 1:80 | 84-106 | 95 |

References

1. Alan E Mast. et al. (2016) *Arterioscler Thromb Vasc Biol.* 36(1):9-14.
2. Jeremy P Wood. et al. (2014) *Blood.* 123(19):2934-43.
3. N L Sanders. et al. (1985) *Blood.* 66(1):204-12.
4. Lwaleed BA. et al. (2006) *J Pathol.* 208(3): 327-39.