

ab108904 – Tissue Factor Pathway Inhibitor (TFPI) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Tissue Factor Pathway Inhibitor (TFPI) in plasma, serum, milk and cerebrospinal fluid.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Tissue Factor Pathway Inhibitor (TFPI) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Tissue Factor Pathway Inhibitor concentrations in plasma, serum, milk, and cerebrospinal fluid.

A Tissue Factor Pathway Inhibitor specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Tissue Factor Pathway Inhibitor specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Tissue Factor Pathway Inhibitor captured in plate.

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. 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. The third Kunitz-type domain and the carboxy-terminus of TFPI mediate its binding to heparin and cell surfaces including the endothelium. 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. The plasma TFPI contains mostly 34 and 40 kDa forms, and the concentration is approximately 50 to 100 ng/mL. 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.

2. ASSAY SUMMARY

Primary capture antibody



Prepare all reagents, samples and standards as instructed.

Sample



Add standard or sample to each well used. Incubate at room temperature.

Primary detector antibody



Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Streptavidin Label



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.

Substrate **Colored product**



Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|-----------|--|
| Tissue Factor Pathway Inhibitor Microplate (12 x 8 well strips) | 96 wells | 4°C |
| Tissue Factor Pathway Inhibitor Standard | 2 vial | 4°C |
| 10X Diluent N Concentrate | 30 mL | 4°C |
| Biotinylated Human Tissue Factor Pathway Inhibitor Antibody | 1 vial | -20°C |
| 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) | 80 µL | -20°C |
| Chromogen Substrate | 7 mL | 4°C |
| Stop Solution | 11 mL | 4°C |
| 20X Wash Buffer Concentrate | 2 x 30 mL | 4°C |
| Sealing Tapes | 3 | N/A |

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 8 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 1X Diluent N

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 1X Wash Buffer

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 1X Biotinylated Tissue Factor Pathway Inhibitor Detector Antibody

9.3.1 The stock Biotinylated Tissue Factor Pathway Inhibitor Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody for use in the assay procedure. Observe the label for the “X” concentration on the vial of Biotinylated Tissue Factor Pathway Inhibitor Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Tissue Factor Pathway Inhibitor Antibody to prepare a 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

ASSAY PREPARATION

| Number of Wells Strips | Number of Wells | (V _T) Total Volume of 1X Biotinylated Antibody (μL) |
|------------------------|-----------------|---|
| 4 | 32 | 1,760 |
| 6 | 48 | 2,640 |
| 8 | 64 | 3,520 |
| 10 | 80 | 4,400 |
| 12 | 96 | 5,280 |

Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Tissue Factor Pathway Inhibitor Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Tissue Factor Pathway Inhibitor Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Tissue Factor Pathway Inhibitor Antibody to prepare 1X Biotinylated Tissue Factor Pathway Inhibitor solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody:

$$V_T - V_A = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Tissue Factor Pathway Inhibitor Antibody stock

C_F = 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody solution for use in the assay procedure

V_T = 3,520 μ L (8 well strips or 64 wells)

$$(1X/50X) \times 3,520 \mu\text{L} = 70.4 \mu\text{L}$$

$$3,520 \mu\text{L} - 70.4 \mu\text{L} = 3,449.6 \mu\text{L}$$

V_A = 70.4 μ L total volume of (X) stock Biotinylated Tissue Factor Pathway Inhibitor Antibody required

V_D = 3,449.6 μ L total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody solution for assay procedures

9.3.3 First spin the Biotinylated Tissue Factor Pathway Inhibitor Antibody vial to collect the contents at the bottom.

9.3.4 Add calculated amount V_A of stock Biotinylated Tissue Factor Pathway Inhibitor Antibody to the calculated amount V_D of 1X Diluent N. Mix gently and thoroughly.

9.4 1X SP Conjugate

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Reconstitute a new vial for each assay.
- This procedure prepares sufficient standard dilutions for duplicate wells.

10.1 Reconstitution of the Tissue Factor Pathway Inhibitor Standard vial to prepare a 10 ng/mL Tissue Factor Pathway Inhibitor **Standard #1**:

10.1.1 First consult the Tissue Factor Pathway Inhibitor Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the Tissue Factor Pathway Inhibitor Standard vial to produce a 10 ng/mL Tissue Factor Pathway Inhibitor **Standard #1** by using the following equation:

C_S = Starting mass of Tissue Factor Pathway Inhibitor Standard (see vial label) (ng)

C_F = 10 ng/mL Tissue Factor Pathway Inhibitor **Stock Standard** final required concentration

V_D = Required volume of 1X Diluent N for reconstitution (μ L)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 18 ng of Tissue Factor Pathway Inhibitor Standard in vial

C_F = 10 ng/mL Tissue Factor Pathway Inhibitor **Standard #1**
final concentration

V_D = Required volume of 1X Diluent N for reconstitution

$$(18 \text{ ng} / 10 \text{ ng/mL}) \times 1,000 = 1,800 \text{ } \mu\text{L}$$

- 10.1.3 First briefly spin the Tissue Factor Pathway Inhibitor Standard Vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the Tissue Factor Pathway Inhibitor Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 10 ng/mL Tissue Factor Pathway Inhibitor **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 10 ng/mL Tissue Factor Pathway Inhibitor **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- 10.3 Label seven tubes #2 – 8.
- 10.4 Add 120 μL of 1X Diluent N to tube #2 – 8.
- 10.5 To prepare **Standard #2**, add 120 μL of the **Standard #1** into tube #2 and mix gently.
- 10.6 To prepare **Standard #3**, add 120 μL of the **Standard #2** into tube #3 and mix gently.
- 10.7 Using the table below as a guide, prepare subsequent serial dilutions.
- 10.8 1X Diluent N serves as the zero standard, 0 ng/mL (tube #8).

ASSAY PREPARATION

Standard Dilution Preparation Table

| Standard # | Volume to Dilute (μL) | Volume Diluent N (μL) | Total Volume (μL) | Starting Conc. (ng/mL) | Final Conc. (ng/mL) |
|------------|------------------------------------|------------------------------------|--------------------------------|------------------------|---------------------|
| 1 | Step 10.1 | | | | 10.00 |
| 2 | 120 | 120 | 240 | 20.00 | 5.000 |
| 3 | 120 | 120 | 240 | 10.00 | 2.500 |
| 4 | 120 | 120 | 240 | 5.000 | 1.250 |
| 5 | 120 | 120 | 240 | 2.500 | 0.625 |
| 6 | 120 | 120 | 240 | 1.250 | 0.313 |
| 7 | 120 | 120 | 240 | 0.625 | 0.156 |
| 8 | - | 120 | 120 | - | 0 |



11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant (EDTA or Heparin can be also be used as an anticoagulant). Centrifuge samples at 3,000 x *g* for 10 minutes. Dilute samples 1:40 with 1X Diluent N and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes and remove serum. Dilute samples 1:40 into 1X Diluent N and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Milk

Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. The sample is suggested for use at 1x or within the range of 2x – 10x into MIX Diluent; Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.4 Cerebrospinal Fluid

Collect cerebrospinal fluid using sample tube. Centrifuge samples at 3,000 x *g* for 10 minutes and assay. The sample is suggested for use at 1x or within the range of 2x – 10x into MIX Diluent. Store samples at -80°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.

ASSAY PREPARATION

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
 - **It is recommended to assay all standards, controls and samples in duplicate.**
- 13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
 - 13.2 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.
 - 13.3 Add 50 μ L of Tissue Factor Pathway Inhibitor Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
 - 13.4 Wash five times with 200 μ L of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 μ L of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
 - 13.5 Add 50 μ L of 1X Biotinylated Tissue Factor Pathway Inhibitor Antibody to each well and incubate for one hour.
 - 13.6 Wash microplate as described above.
 - 13.7 Add 50 μ L of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 13.8 Wash microplate as described above.

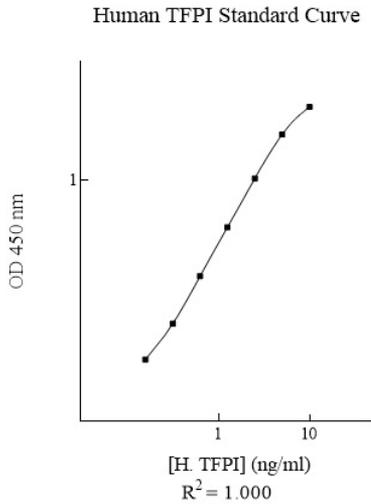
- 13.9 Add 50 μL of Chromogen Substrate per well and incubate in ambient light for about 20 minutes or till the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- 13.10 Add 50 μL of Stop Solution to each well. The color will change from blue to yellow.
- 13.11 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 some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

14. CALCULATIONS

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.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Tissue Factor Pathway Inhibitor is typically ~52 pg/mL.

Spiking RECOVERY –

Recovery was determined by spiking one plasma and one serum sample with different TFPI concentrations.

Recovery %: 91 - 108.

Average Recovery %: 99.

| Sample | Unspiked Sample (ng/ml) | Spiking Value (ng/ml) | Expected | Observed | Recovery (%) |
|-----------------------------|-------------------------|-----------------------|----------|----------|--------------|
| Plasma | 1.682 | 0.861 | 2.543 | 2.747 | 108% |
| | | 0.438 | 2.120 | 2.188 | 103% |
| | | 0.239 | 1.921 | 1.739 | 91% |
| Serum | 1.388 | 0.861 | 2.249 | 2.220 | 99% |
| | | 0.438 | 1.826 | 1.772 | 97% |
| | | 0.239 | 1.627 | 1.600 | 98% |
| Average Recovery (%) | | | | | 99% |

LINEARITY OF DILUTION –

| Plasma Dilution | Average % Expected Value |
|-----------------|--------------------------|
| 1:20 | 90 |
| 1:40 | 102 |
| 1:80 | 110 |

| Serum Dilution | Average % Expected Value |
|----------------|--------------------------|
| 1:20 | 91 |
| 1:40 | 99 |
| 1:80 | 110 |

PRECISION –

| | Intra-Assay | Inter-Assay |
|------|-------------|-------------|
| % CV | 3.5 | 10.4 |

17. ASSAY SPECIFICITY

| Species | % Cross Reactivity |
|---------|--------------------|
| Canine | None |
| Bovine | None |
| Equine | 0.5 |
| Monkey | <15 |
| Mouse | None |
| Rat | None |
| Swine | <10 |
| Rabbit | None |

18. TROUBLESHOOTING

| Problem | Cause | Solution |
|---------------------|--|---|
| Poor standard curve | Improper standard dilution | Confirm dilutions made correctly |
| | Standard improperly reconstituted (if applicable) | Briefly spin vial before opening; thoroughly resuspend powder (if applicable) |
| | Standard degraded | Store sample as recommended |
| | Curve doesn't fit scale | Try plotting using different scale |
| Low signal | Incubation time too short | Try overnight incubation at 4°C |
| | Target present below detection limits of assay | Decrease dilution factor; concentrate samples |
| | Precipitate can form in wells upon substrate addition when concentration of target is too high | Increase dilution factor of sample |
| | Using incompatible sample type (e.g. serum vs. cell extract) | Detection may be reduced or absent in untested sample types |
| | Sample prepared incorrectly | Ensure proper sample preparation/dilution |

RESOURCES

| Problem | Cause | Solution |
|----------------|--|---|
| Large CV | Bubbles in wells | Ensure no bubbles present prior to reading plate |
| | All wells not washed equally/thoroughly | Check that all ports of plate washer are unobstructed wash wells as recommended |
| | Incomplete reagent mixing | Ensure all reagents/master mixes are mixed thoroughly |
| | Inconsistent pipetting | Use calibrated pipettes and ensure accurate pipetting |
| | Inconsistent sample preparation or storage | Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles) |

| Problem | Cause | Solution |
|-------------------------------------|--|--|
| High background/ Low sensitivity | Wells are insufficiently washed | Wash wells as per protocol recommendations |
| | Contaminated wash buffer | Make fresh wash buffer |
| | Waiting too long to read plate after adding STOP solution | Read plate immediately after adding STOP solution |
| | Improper storage of ELISA kit | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
| | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

19. NOTES

Technical Support

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For all technical or commercial enquiries please go to:

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