

**APA394Hu02 100µg**  
**Active Tissue Factor Pathway Inhibitor (TFPI)**  
**Organism Species: *Homo sapiens* (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Asp29~Met304

**Tags:** N-terminal His and GST Tag

**Purity:** >80%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .

**Original Concentration:** 200µg/mL

**Applications:** Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 7.9

**Predicted Molecular Mass:** 61.9kDa

**Accurate Molecular Mass:** 60kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

## **[ STORAGE AND STABILITY ]**

**Storage:** Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## [ **SEQUENCE** ]

```
DS EDEEHTIIT DTELPPLKLM
HSFCAFKADD GPCKAIMKRF FFNIFTRQCE EFIYGGCEGN QNRFESLEEC
KKMCTRDNAN RIIKTTLQQE KPDFCFLEED PGICRGYITR YFYNNQTKQC
ERFKYGGCLG NMNNFETLEE CKNICEDGPN GFQVDNYGTQ LNAVNNSLTP
QSTKVP SLFE FHGPSWCLTP ADRGLCRANE NRFYNSVIG KCRPFKYSGC
GGNENNFTSK QECLRACKKG FIQRISKGGL IKTKRKRKKQ RVKIAVEEIF
VKNM
```

## [ **ACTIVITY** ]

Human TFPI, also known as lipoprotein-associated coagulation inhibitor (LACI) and extrinsic pathway inhibitor (EPI), is a physiological inhibitor of extrinsic pathway of coagulation and has biological functions of anticoagulation and anti-inflammation. It is a secreted protein with a N-terminal acidic region, three Kunitz (K) domains separated with by two linker regions, and a C-terminal basic region. The activity of recombinant human TFPI was measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate Mca-RPKPVE-Nval-WRK(Dnp)-NH<sub>2</sub> in the assay buffer 50 mM Tris, 10 mM CaCl<sub>2</sub>, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5. Trypsin was diluted to 50 ug/ml in the assay buffer and 20 ul different concentrations of recombinant human TFPI (MW: 61.9 KD) was incubated with 20 ul diluted trypsin at 37 °C for 15 minutes. Loading 50 µL of the incubated mixtures which were diluted five-fold in assay buffer into empty wells of a plate, and start the reaction by adding 50 µL of 20 µM substrate. Include a substrate blank containing 50 µL of assay buffer and 50 µL of 20 µM substrate. Then read at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The result was shown in Figure 1

and it was obvious that recombinant human TFPI significantly decreased trypsin activity. The inhibition IC<sub>50</sub> was <3 nM.

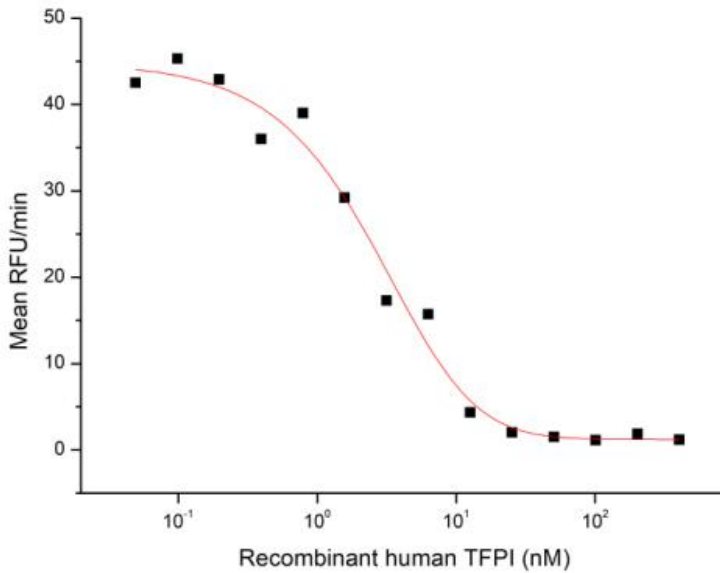


Figure 1. Inhibition of trypsin activity by recombinant human TFPI

## [ IDENTIFICATION ]

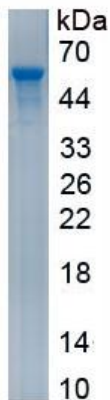


Figure 2. SDS-PAGE

Sample: Active recombinant TFPI, Human

## [ IMPORTANT NOTE ]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.