APA394Ra01 100μg
Active Tissue Factor Pathway Inhibitor (TFPI)
Organism Species: *Rattus norvegicus (Rat)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Leu29~His302
Tags: N-terminal His-tag
Purity: >90%
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: 8.2
Predicted Molecular Mass: 32.7kDa
Accurate Molecular Mass: 36kDa 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 2-8ºC for one month.
Aliquot and store at -80ºC 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 37ºC 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]

LP EEDDDTINTD SELRPMKPLH
TFCAMKAEDG PCKAMIRSYY FNMNSHQCEE FIYGGCRGNK NRFDTLEECR
KTCIPGYKKT TIKTTSGAEK PDFCFLEEDP GICRGFMTRY FYNNQSKQCE
QFKYGGCLGN SNNFETLEEC RNTCEDPVNE VQKGDYVTNQ ITVTDRTTVN
NVVIPQATKA PSQWDYDGPS WCLEPADSGL CKASEKRFYY NPAIGKCRQF
NYTGCGGNNN NFTTKQDCNR ACKKDSSKKS SKRAKTQRRR KSFVKVMYEN
IH

[ACTIVITY]

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 rat TFPI was measured by its ability to inhibit trypsin cleavage of a fluorogenic peptide substrate Mca-RPKPVE-Nval-WRK(Dnp)-NH2 in the assay buffer 50 mM Tris, 10 mM CaCl2, 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 rat TFPI (MW: 61.9 KD) was incubated with 20 ul diluted trypsin at 37 ℃ 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 excitiation 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 rat TFPI significantly decreased trypsin activity. The inhibition IC50 was <10 nM.

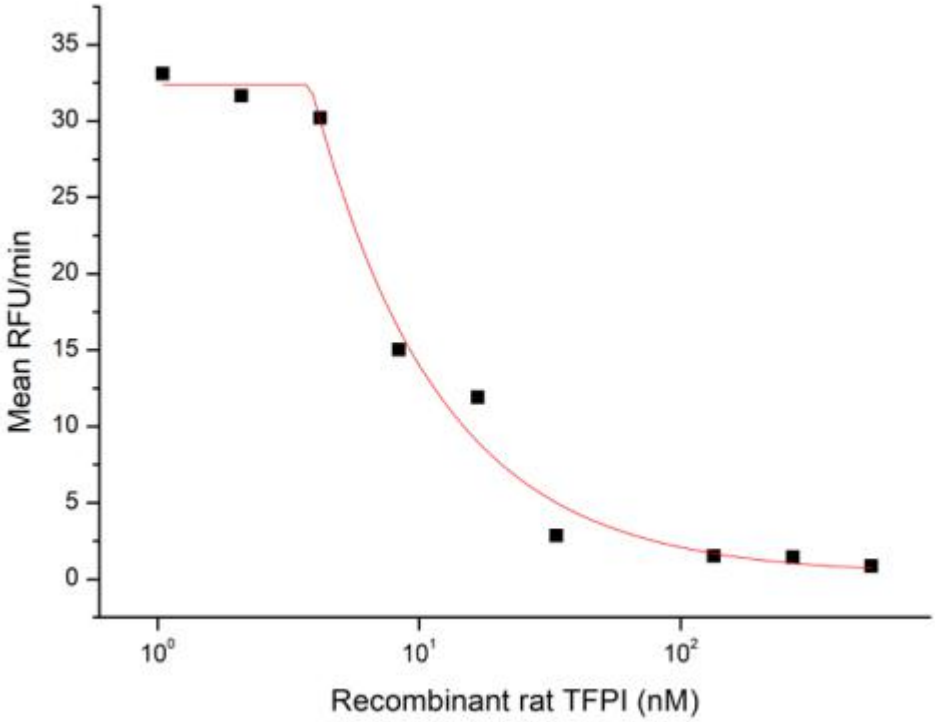

Figure 1. Inhibition of trypsin activity by recombinant rat TFPI

[IDENTIFICATION]

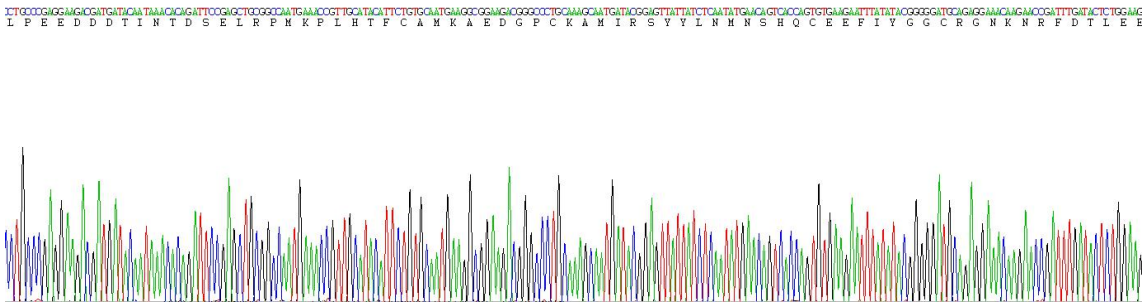

Figure 2. Gene Sequencing (extract)

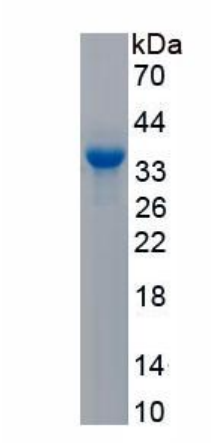

Figure 3. SDS-PAGE

Sample: Active recombinant TFPI, Rat

[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.