APB940Hu01 10μg
Active Tissue Factor Pathway Inhibitor 2 (TFPI2)
Organism Species: *Homo sapiens (Human)*
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: Asp23~Phe235
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5% Trehalose.
Original Concentration: 50μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 8.9
Predicted Molecular Mass: 25.9kDa
Accurate Molecular Mass: 30kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in ddH_2O to a concentration of 0.1-0.2 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.

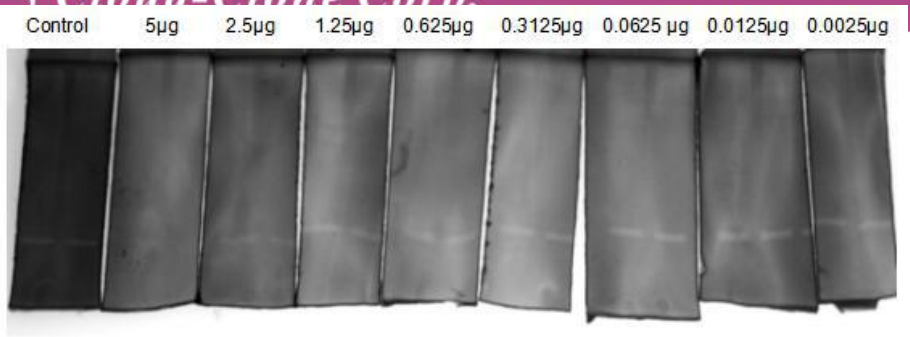
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[SEQUENCE]

DAAQEPTG NNAEICLLPL DYGPCRALLL
RYYYDRYTQS CRQFLYGGCE GNANNFYTWE ACDDACWRIE KVPKVCRLQV
SVDDQCEGST EKYFFNLSSM TCEKFFSGGC HRNRIENRFP DEATCMGFCA
PKKIPSFCYS PKDEGLCSAN VTRYYFNPRY RTCDAFTYTG CGGNDNNFVS
REDCKRACAK ALKKKKKMPK LRFASRIRKI RKKQF

[ACTIVITY]

Tissue Factor Pathway Inhibitor 2 (TFPI2) takes part in the regulation of plasmin-mediated matrix remodeling. Inhibits trypsin, plasmin, factor VIIa/tissue factor and weakly factor Xa. TFPI2 does not have any influence on thrombin. TFPI2 also can inhibit MMP activity, which can hydrolyze gelatin under certain conditions. Thus, the activity of TFPI2 can be measured by inhibit MMP-2 hydrolyze gelatin. Gelatin zymography is mainly used for the detection of the gelatinases, 2μg/mL was denatured by SDS loading buffer, electrophoresed through sodium dodecylsulphate–polyacrylamide gel (SDS–PAGE; 8%gels) containing gelatin (1mg/mL) with nonreducing conditions. After renaturation, incubate with various concentrations of recombinant human TFPI2, then staining with coomassie brilliant blue G250, active MMP-2 would hydrolyze gelatin nearby, which was indicated by the white binds on the gel; if the activity of MMP-2 inhibit by TFPI2, there was none white binds on the gel. The result was shown in figure 1.



As the figure1 shown, MMP-2 can be inhibited by recombinant human TFPI2 at least 5μg/ml.

The activity of recombinant human TFPI2 was also 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 human TFPI2 (MW: 25.89 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 2 and it was obvious that recombinant human TFPI2 significantly decreased trypsin activity. The inhibition IC50 was <60 nM.

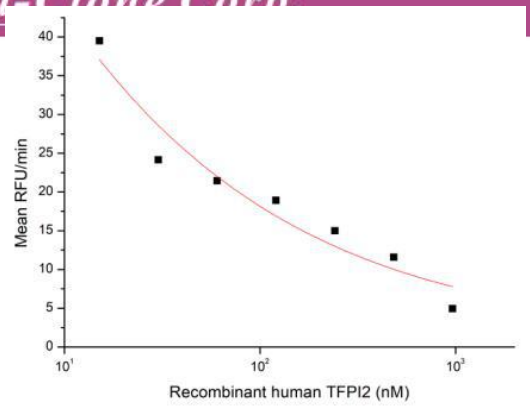


Figure 2. Inhibition of trypsin activity by recombinant human TFPI2

[IDENTIFICATION]

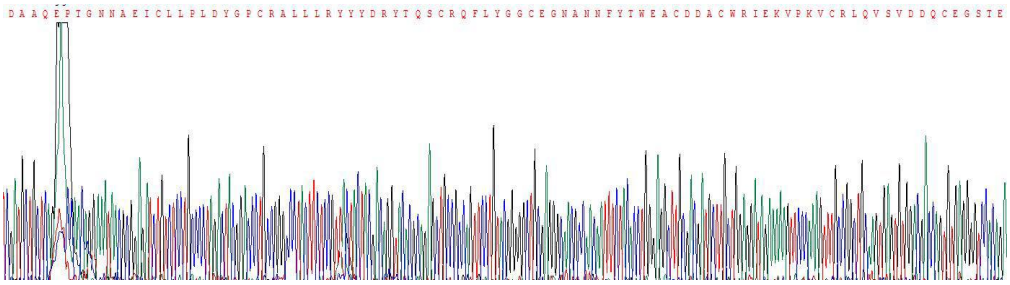


Figure 3. Gene Sequencing (extract)

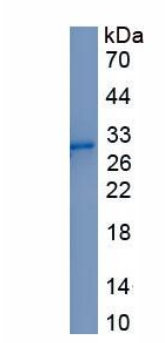


Figure 4. SDS-PAGE

Sample: Active recombinant TFPI2, 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.