

APA525Hu61 100µg
Active Tissue Plasminogen Activator (tPA)
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: Eukaryotic expression.

Host: 293F cell

Residues: Ile311~Pro562

Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: PBS, pH7.4, containing 5% trehalose.

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.5

Predicted Molecular Mass: 29.7kDa

Accurate Molecular Mass: 33-38kDa 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 10mM PBS (pH7.6) 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.

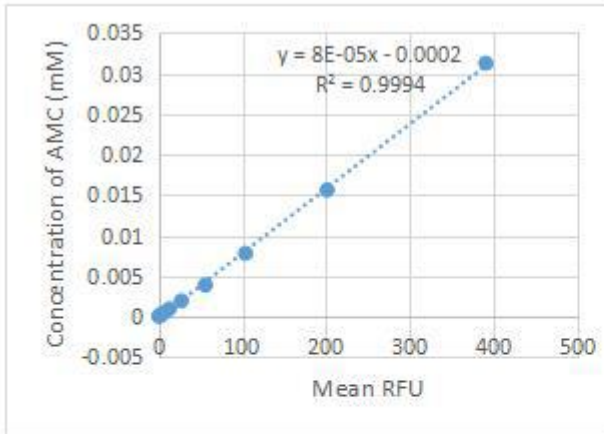
[SEQUENCE]

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IKGGLFADIA SHPWQAAIFA KHRRSPGERF LCGGILISSC  
WILSAAHCFQ ERFPPHLLTV ILGRTYRVVP GEEEQKFEVE KYIVHKEFDD  
DTYDNDIALL QLKSDSSRCA QESSVRTVC LPPADLQLPD WTECELSGYG  
KHEALSPFYS ERLKEAHVRL YPSSRCTSQH LLNRTVTDNM LCAGDTRSGG  
PQANLHDACQ GDSGGPLVCL NDGRMTLVGI ISWGLGCGQK DVPGVYTKVT  
NYLDWIRDNM RP
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[ACTIVITY]

Plasminogen activators are serine proteases that catalyze the activation of plasmin via proteolytic cleavage of its zymogen form plasminogen. Plasmin is an important factor in fibrinolysis, the breakdown of fibrin polymers formed during blood clotting. There are two main plasminogen activators: urokinase (uPA) and tissue plasminogen activator (tPA). Tissue plasminogen activators (tPA) are used to treat medical conditions related to blood clotting including embolic or thrombotic stroke, myocardial infarction, and pulmonary embolism. The activity assay of tPA was measured by its ability to cleave a peptide substrate, N-carbobenzyloxy-Gly-Gly-Arg-7-amido-4-methylcoumarin (Z-GGR-AMC). The

reaction was performed in 50 mM Tris, 0.01% Tween-20, pH 8.5 (Assay Buffer), initiated by addition 50 µL of various concentrations of tPA (diluted by Assay Buffer) to 50 µL of 200 uM Substrate. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human tPA is 10-60 pmol/min/µg.



RFU	AMC (mM)
390.1509	0.03125
201.1509	0.015625
103.9509	0.0078125
56.0009	0.00390625
27.4709	0.001953125
13.1709	0.000976563
6.5189	0.000488281
3.0259	0.000244141
1.4129	0.00012207
0.7129	6.10352E-05

Figure 1. The standard curve of AMC

One unit of enzyme activity is defined as the 1 µg of enzyme required to convert 1 pmol of Z-GGR-AMC to AMC in 1min.

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\Delta OD * F}{T * N}$$

ΔOD=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of AMC)

T= Time

N=Amount of enzyme

[IDENTIFICATION]

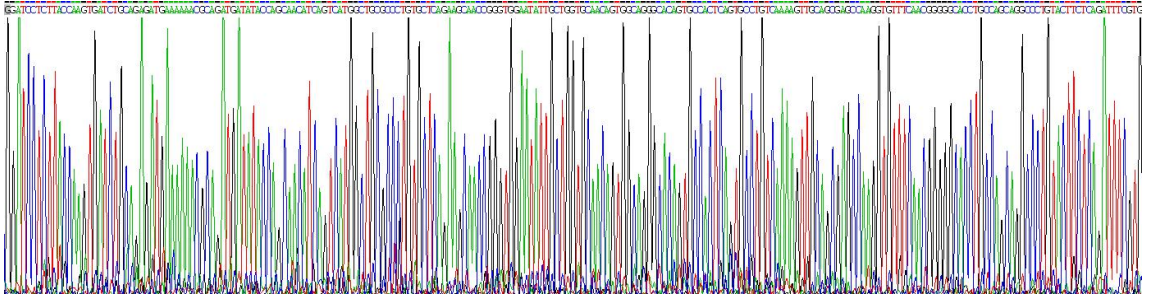


Figure 2. Gene Sequencing (extract)

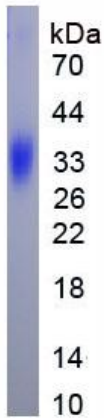


Figure 3. SDS-PAGE

Sample: Active recombinant tPA, 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.