APA140Mu61 100μg
Active Plasminogen Activator, Urokinase (uPA)
Organism Species: *Mus musculus (Mouse)*
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: Gly21~Phe433
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 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: 8.5
Predicted Molecular Mass: 48.3kDa
Accurate Molecular Mass: 27&19kDa 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 0.1-0.5 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]

GSVLGAPDESNCGCQNGGVCVSYKYFSRIRRCSCPRKFQGEHCEIDASKTCYHGNGDSYRGKANTDTKG
RPCLAWNAPAVLQKPYNAHRPDAISLGLGKHNYCRNPDNQKRPWCYVQIGLRQFVQECMVHDCSLSKKP
SSSVDQQGFQCGQKALRPRFKIVGGEFTEVENQPWFAAIYQKNKGGSPPSFKCGGSLISPCWVASAAHC
FIQLPKKENYVVYLGQSKESSYNPGEMKFEVEQLILHEYYREDSLAYHNDIALLKIRTSTGQCAQPSRS
IQTICLPPRFTDAPFGSDCEITGFGKESESDYLYPKNLKMSVVKLVSHEQCMQPHYYGSEINYKMLCAA
DPEWKTDSCKGDSGGPLICNIEGRPTLSGIVSWGRGCAEKNKPGVYTRVSHFLDWIQSHIGEEKGLAF

[ACTIVITY]

Urokinase Plasminogen Activator (uPA), also known as u-plasminogen activator or urokinase, is a highly-specific serine protease from the peptidase S1 family that cleaves plasminogen to form plasmin making it a key player in the plasminogen activator (PA) system. Expression of uPA is minimal in normal cells but is increased several fold in tumor cells by extracellular stimuli elevated in cancer and corresponds to poor outcomes in several types of cancer. Therefore, uPA has been identified as an excellent target for therapeutic development through inhibition of protease activity or though inhibition of uPA-dependent signaling while in complex with uPA receptor (uPAR).

The activity assay of uPA 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), ainitiated by addition 50 μL of 1.5 ug/ml uPA (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 mouse uPA is >2000 pmol/min/μg.

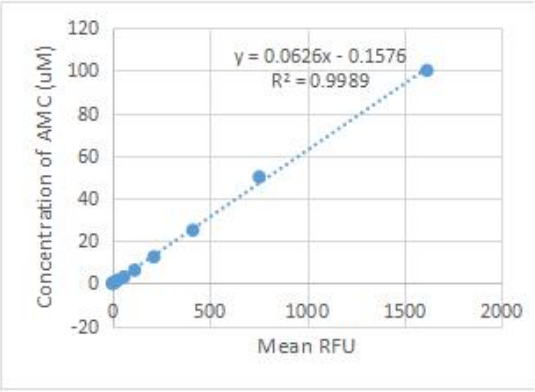

Figure 1. The standard curve of AMC

RFU	AMC (uM)
1616.7009	100
754.1009	50
413.2009	25
212.8009	12.5
113.8009	6.25
58.9809	3.125
27.7509	1.5625
14.9209	0.78125
7.9949	0.390625
3.7939	0.1953125
1.8779	0.09765625
0.9849	0.048828125
0.441	0.024414063

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.

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

△OD=Adjusted for Substrate Blank

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

T= Time

N=Amount of enzyme

[IDENTIFICATION]

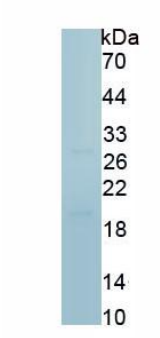

Figure 2. SDS-PAGE

Sample: Active recombinant uPA, Mouse

[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.