Recombinant Human u-Plasminogen Activator (uPA)/Urokinase

Catalog Number: 1310-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human u-Plasminogen Activator (uPA)/Urokinase protein Met1-Leu431 with a C-terminal 10-His tag Accession # P00749
N-terminal Sequence Analysis	Ser21, Ile179 & Lys156
Predicted Molecular Mass	18 kDa (long A chain), 3 kDa (short A chain), 30 kDa (B chain)

SPECIFICATIONS

SDS-PAGE	18 kDa and 32 kDa, reducing conditions
Activity	Measured by its ability to cleave a peptide substrate, N-carbobenzyloxy-Gly-Gly-Arg-7-amido-4-methylcoumarin (Z-GGR-AMC). The specific activity is >2,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.01% (v/v) Tween® 20, pH 8.5
- Recombinant Human u-Plasminogen Activator (uPA)/Urokinase (rhuPA) (Catalog # 1310-SE)
- Substrate: Z-Gly-Gly-Arg-AMC (Bachem, Catalog # I-1140), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhuPA to 1 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load 50 µL of the 1 ng/µL rhuPA into a black well plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM Substrate without any rhuPA.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

Final Assay Conditions

Per Well:

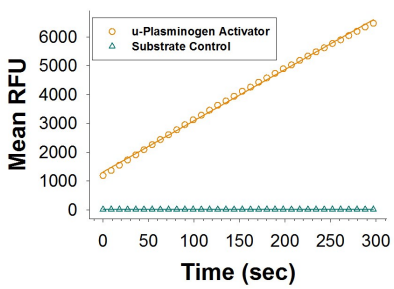
- rhuPA: 0.05 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

Enzyme Activity



Recombinant Human u-Plasminogen Activator/Urokinase (Catalog # 1310-SE) is measured by its ability to cleave a peptide substrate, N-carbobenzyloxy-Gly-Gly-Arg-7-amido-4-methylcoumarin (Z-GGR-AMC).

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BACKGROUND

uPA is a serine protease with an extremely limited substrate specificity, cleaving the sequence Cys-Pro-Gly-Arg560-Val561-Val-Gly-Gly-Cys in plasminogen to form plasmin (1). uPA is a potent marker of invasion and metastasis in a variety of human cancers associated with breast, stomach, colon, bladder, ovary, brain and endometrium (2). For example, the combination (both low vs. either or both high) of uPA and its inhibitor, plasminogen activator inhibitor-1 (PAI-1), outperforms the single factors as well as other traditional prognostic factors with regard to risk group assessment for breast cancer, particularly in node-negative breast cancer (3). The human uPA is initially synthesized as 431 amino acid precursor with a N-terminal signal peptide (20 residues) (4-6). The single chain molecule is processed into a disulfide-linked two-chain molecule. The B chain starting at Ile179 corresponds to the catalytic domain. Two forms of the A chain exist, one starting at Ser21 (the long form) and the other at Lys156 (the short form). The resulting two-chain forms have different molecular weights (MW). The B chain is common for both forms whereas the long and short A chains are unique to the high and low MW forms, respectively. The long A chain contains an EGF-like domain, which is responsible for binding of the uPA receptor (uPAR). Both high and low MW forms exist in the purified recombinant human uPA.

References:

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