Recombinant Mouse u-Plasminogen Activator (uPA)/Urokinase His-tag

Catalog Number: 11143-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse u-Plasminogen Activator (uPA)/Urokinase protein Gly21-Phe433, with a C-terminal 6-His tag Accession # NP_032899.1
N-terminal Sequence Analysis	Lys157, Ile180, Met361
Predicted Molecular Mass	18 kDa (long A chain), 3 kDa (short A chain), 29 kDa (B chain)

SPECIFICATIONS

SDS-PAGE	24-33 kDa (short form), under non-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 >1700 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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 Mouse u-Plasminogen Activator (uPA)/Urokinase (rmuPA) (Catalog # 11143-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 M5 by Molecular Devices) or equivalent

Assay

1. Dilute rmuPA to 1 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Load 50 µL of 1 ng/µL rmuPA into a black well plate, and start the reaction by adding 50 µL of 2 mM Substrate. Include a Substrate Blank containing 50 µL of Assay buffer and 50 µL of 2 mM Substrate.
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 from calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A9891)

Final Assay Conditions

Per Well:

- rmuPA: 0.05 µg
- Substrate: 1 mM

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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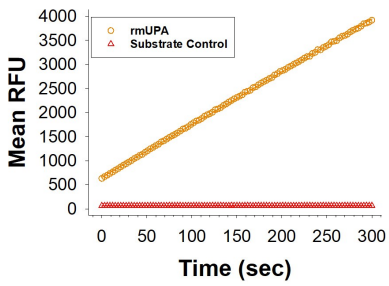
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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 Mouse u-Plasminogen Activator (uPA)/Urokinase His-tag Protein Enzyme Activity. Recombinant Mouse u-Plasminogen Activator (uPA)/Urokinase His-tag (Catalog # 11143-SE) is measured by its ability to cleave a peptide substrate, N-carbobenzyloxy-Gly-Gly-Arg-7-amido-4-methylcoumarin (Z-GGR-AMC).

BACKGROUND

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 (1, 2). In cancer, the PA system plays a commanding role in tumor growth, angiogenesis, tumor cell invasion, migration, and metastasis. Expression of uPA is minimal in normal cells but is increased several fold in tumor cells by extracellular stimuli elevated in cancer (3) and corresponds to poor outcomes in several types of cancer (2, 4-7). 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) (2, 7). The pro-enzyme of uPA is synthesized with an N-terminal signal peptide and processed into an active disulfide-linked two-chain molecule (2, 7-10). For mouse uPA, the B chain starting at Ile180 corresponds to the catalytic domain. Two forms of the A chain exist, one starting at Gly21 (the long form) and the other at Lys157 (the short form). While the B chain is common for both forms, the long and short A chains are unique to expected 47 kDa and 32 kDa two-chain forms, respectively. The long A chain contains an EGF-like domain and the kringle domain. The long A domain is reported as responsible for the binding of the uPA receptor (uPAR) (2, 7).

References:

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