Biotinylated Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag

Catalog Number: AVI1310

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human u-Plasminogen Activator (uPA)/Urokinase protein Met1-Leu431 with C-terminal 6-His and Avi-tag Accession # P00749.2
N-terminal Sequence Analysis	Lys156 & Phe177
Predicted Molecular Mass	18 kDa (long A chain), 3 kDa (short A chain), 31 kDa (B chain)

SPECIFICATIONS

SDS-PAGE	19-20 kDa & 32-38 kDa, under 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 >2000 pmol/min/µg, as measured under the described conditions. Measured by its binding ability in a functional ELISA. Biotinylated Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag (Catalog # AVI1310) binds to Human u-Plasminogen Activator (uPA) Antibody (Catalog # MAB1310) with an ED_{50} of 0.300-4.50 ng/mL.
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
- Biotinylated Recombinant Human u-Plasminogen Activator/Urokinase His-tag Avi-tag (rhuPA) (Catalog # AVI1310)
- Substrate: Z-GGR-AMC, 10 mM stock in DMSO
- Black 96-Well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhuPA to 0.5 µg/mL in Assay Buffer.
2. Dilute Substrate to 400 µM in Assay Buffer.
3. Load 50 µL of the 0.5 µg/mL rhuPA into a black well plate and start the reaction by adding 50 µL of 400 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 400 µM 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 using calibration standard 7-amino, 4-Methyl Coumarin (AMC)

Final Assay Conditions

Per Well:

- rhuPA His Avi: 0.025 µg
- Substrate: 200 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

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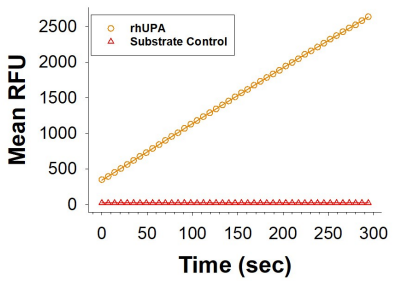
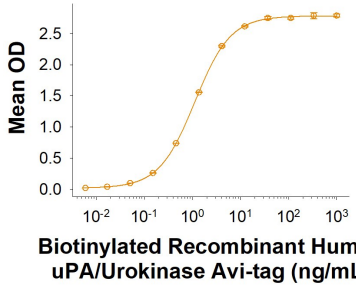
Enzyme Activity



Biotinylated Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag Protein Enzyme Activity. Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag (Catalog # AVI1310) is measured by its ability to cleave a peptide substrate, N-carbobenzyloxy-Gly-Gly-Arg-7-amido-4-methylcoumarin (Z-GGR-AMC).

Binding Activity



Biotinylated Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag Protein Binding Activity. In a functional ELISA, Biotinylated Recombinant Human u-Plasminogen Activator (uPA)/Urokinase His-tag Avi-tag Protein (Catalog # AVI1310) binds to Human u-Plasminogen Activator (uPA) Antibody (Catalog # MAB1310) with an ED_{50} of 0.300-4.50 ng/mL

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 a N-terminal signal peptide and processed into an active disulfide-linked two-chain molecule (2, 7-10). For human uPA, 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). While the B chain is common for both forms, the long and short A chains are unique to expected 49 kDa and 34 kDa two-chain forms, respectively. The long A chain contains an EGF-like domain and the kringle domain. The long A domain is reportedly responsible for the binding of the uPA receptor (uPAR) (2,7).

References:

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