Recombinant Human t-Plasminogen Activator/tPA

Catalog Number: 7449-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Met1-Pro562, with a C-terminal 6-His tag Accession # P00750 Single-chain proform was expressed, purified, activated with thermolysin to a 2-chain protease and further purified.
N-terminal Sequence Analysis	Ser36 (Chain A) and Ile311 (Chain B)
Predicted Molecular Mass	31 kDa (Chain A), 29 kDa (Chain B)

SPECIFICATIONS

SDS-PAGE	30-40 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 >190 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	>85%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.01% Tween-20, pH 8.5
- Recombinant Human t-Plasminogen Activator/tPA (rhPLAT) (Catalog # 7449-SE)
- Fluorogenic Peptide 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 rhPLAT to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load 50 µL of the 2 ng/µL rhPLAT 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 rhPLAT.
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) (Sigma, Catalog # A9891).

Final Assay Conditions

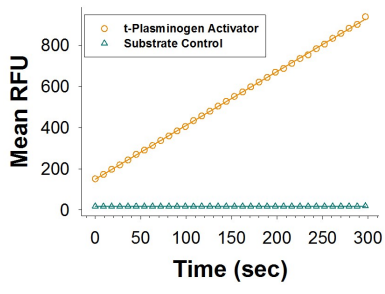
- rhPLAT: 0.1 µg
- Substrate: 100 µM

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 t-Plasminogen Activator (Catalog # 7449-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

PLAT, also known as tissue-type plasminogen activator (tPA), is a secreted serine protease synthesized by endothelial cells (1). The partially active single chain can be further processed to full activity by plasmin, tissue kallikrein or Factor Xa. Active PLAT converts plasminogen to plasmin, a fibrinolytic protease, by hydrolyzing an Arg-Val peptide bond in plasminogen. Unusually high levels of tPA activity can result in excessive bleeding, and low levels of tPA activity can result in thrombosis or embolism. Human PLAT contains 4 domains; the N-terminal fibronectin type-1 domain, an epidermal growth factor-like domain, two kringle domains and a serine protease catalytic domain.

References:

1. Lijnen H.R. and D. Collen (2004) Handbook of Proteolytic Enzymes (ed. Barrett, *et al.*) p. 1684, Academic Press, San Diego.

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