**DESCRIPTION**

**Source**

Plasminogen protein

The human plasma used for the isolation of this product was certified by the supplier to be HIV-1 and HBsAg negative at the time of shipment. Human blood products should always be treated in accordance with universal handling precautions.

**N-terminal Sequence Analysis**

EPLDDYNTQ (major) and LDDYNVTQGA (minor)

**SPECIFICATIONS**

**SDS-PAGE**  
100-106 kDa doublet, reducing conditions

**Activity**

Measured by its ability to cleave the fluorogenic peptide substrate, SUC-AFK-AMC. The specific activity, measured under the described conditions, is >500 pmol/min/µg.

**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**

Lyophilized from a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- Activation Buffer: 50 mM Tris, 0.01% Tween® 20, pH 8.5
- Assay Buffer: 0.1 M Tris, 0.1 M NaCl, pH 7.5
- Human Plasminogen (hPLG) (Catalog # 1939-SE)
- Recombinant Human u-Plasminogen Activator (uPA)/Urokinase (rhuPA) (Catalog # 1310-SE)
- Substrate: SUC-Ala-Phe-Lys-AMC (Bachem, Catalog # I-1330), 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 hPLG to 200 µg/mL in Activation Buffer.
2. Dilute rhuPA to 4 µg/mL in Activation Buffer.
3. Combine 25 µL of diluted hPLG with 25 µL of diluted rhuPA for final concentrations of 100 µg/mL and 2 µg/mL respectively.
4. Incubate at 37 °C for 15 minutes.
5. Dilute activated hPLG to 2 ng/µL in Assay Buffer.
6. Dilute Substrate to 200 µM in Assay Buffer.
7. Load 50 µL of 2 ng/µL hPLG in a black well plate, and start the reaction by adding 50 µL of 200 µM substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 200 µM Substrate.
8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
9. Calculate specific activity:

\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times \text{ Conversion Factor} \times \text{Amount of enzyme (µg)}}{\text{RFU/min}}
\]

*Adjusted for Substrate Blank**

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

**Final Assay Conditions**

Per Well:

- hPLG: 0.1 µg
- Substrate: 100 µM

**PREPARATION AND STORAGE**

**Reconstitution**

Reconstitute at 1 mg/mL in sterile 25 mM Tris and 150 mM NaCl, pH 8.0.

**Shipping**

The product is shipped at ambient temperature. 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 reconstitution.

**BACKGROUND**

Plasminogen (PLG) is the precursor of plasmin, an active serine protease that dissolves the fibrin of blood clots and acts in many other processes such as embryonic development, tissue remodeling, inflammation and tumor invasion (1, 2). Synthesized in the kidney, PLG is found in plasma and many extracellular fluids. Activated by u- or t-plasminogen activator, the single-chain PLG (amino acid residues 20-810) is converted to plasmin, which consists of disulfide bond-linked heavy chain A (residues 20-580) and light chain B (residues 581-810). Heavy chain A contains 5 kringle domains and light chain B corresponds to the serine protease domain. A fragment consisting of the first 4 kringle domains has been named as angiostatin, a novel angiogenesis inhibitor (3, 4).

**References:**