3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Tissue plasminogen activator chromogenic substrate

Product Number **T2943**
Storage Temperature 2-8 °C

Product Description

Formula: CH_3SO_2-D-HHT-Gly-Arg-pNA•AcOH
HHT – hexahydrotyrosine
pNA – p-nitroanilide
Molecular Weight: 658.9

This product is a highly sensitive chromogenic peptide substrate for tissue type plasminogen activator (tPA). Tissue plasminogen activator chromogenic substrate shows different sensitivity to native single-chain tPA (sc-tPA) and its fully active two-chain form (tc-tPA).[1]

Tissue plasminogen activator is a fibrinolytic serine protease, which has been found in many tissues and body fluids. tPA is known to convert plasminogen to plasmin, which then dissolves fibrin, a major component of blood clots. This proteolytic activity results in the hydrolysis of the substrate and release of free p-nitroaniline.

Precautions and Disclaimer

This product is for laboratory use only. Please consult the Material Data Safety Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The product is soluble in water (4 mM).

Storage/Stability

It is recommended to store the product at 2-8 °C.

Procedure

The proteolytic reaction of sc-tPA or tc-tPA with tissue plasminogen activator chromogenic substrate releases free p-Nitroaniline (pNA) in solution. Determination of the free pNA is performed spectrophotometrically by monitoring the ΔA/minute at 405 nm, using a millimolar extinction coefficient (E^{mM}) of 8.80.

The reaction with sc-tPA has a K_M of 0.286 mM and an observed V_{max} of 6.95 nmole/µg of tPA/minute. The reaction may be performed in a disposable cuvette with the following suggested conditions for the determination of sc-tPA activity:

1.78 ml of ~30 mM Tris-HCl, pH 8.4, with ~30 mM imidazole and ~130 mM NaCl
0.02 ml of sc-tPA solution (approximately 78,000 IU/ml)
0.20 ml of chromogenic substrate solution (2 mM in distilled water).

Mix the reaction solution by inversion and measure the ΔA/minute at 405 nm. The reaction has a linear rate for at least 5 minutes.

The reaction with tc-tPA has a K_M of 0.167 mM and an observed V_{max} of 33.9 nmole/µg of tPA/minute. The reaction may be performed in a disposable cuvette with the following suggested conditions for the determination of tc-tPA activity:

1.75 ml of ~30 mM Tris-HCl, pH 8.4, with ~30 mM imidazole and ~130 mM NaCl
0.05 ml of tc-tPA solution (approximately 121,000 IU/ml)
0.20 ml of chromogenic substrate solution (4 mM in distilled water).

Mix the reaction solution by inversion and measure the ΔA/minute at 405 nm. The reaction has a linear rate for at least 5 minutes.

References

1. Tissue Plasminogen Activators in Thronbolytic Therapy: Physiological and Clinical Aspects, Kluft, C., Vols. 1 and 2, CRC Press, (Boca Raton, FL: 1988), p.47.
2. Bachmann, F., and Kruithof, E., Tissue plasminogen activators: chemical and physiological aspects. Semin. Thromb. Hemos., **10**(1), 6-17 (1984).

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