Recombinant Human
Complement Factor D/Adipsin
Catalog Number: 1824-SE

DESCRIPTION

Source: Mouse myeloma cell line, NS0-derived Ile26-Ala253, with a C-terminal 10-His tag
Accession # P00746

N-terminal Sequence Analysis: Ile26 & His35

Predicted Molecular Mass: 26 kDa & 25 kDa

SPECIFICATIONS

SDS-PAGE: 27 kDa & 26 kDa, reducing conditions


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: Supplied as a 0.2 µm filtered solution in Tris, NaCl, CaCl₂ and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials:
- Assay Buffer: 50 mM Tris, 1 M NaCl, pH 7.5
- Recombinant Human Complement Factor D/Adipsin (rhFactor D) (Catalog # 1824-SE)
- Substrate: Z-Lys-SBzl (Bachem, Catalog # M1300), 10 mM stock in DMSO
- 5,5'Dithio-bis-(2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D8130), 10 mM stock in DMSO
- 96 Well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay:
1. Dilute rhFactor D to 5 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer with 200 µM DTNB.
3. Load 50 µL of the diluted rhFactor D into a clear plate, and start the reaction by adding 50 µL of Substrate/DTNB mixture to wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate mixture without any rhFactor D.
4. Read in kinetic mode for 20 minutes at an absorbance of 405 nm.
5. Calculate specific activity:

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\text{Specific Activity (pmol/min/µg) = } \frac{\text{Adjusted } V_{\text{max}} \times (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol ext. coeff} \times (\text{M}^{-1} \text{cm}^{-1}) \times \text{path corr.} \times (\text{cm}) \times \text{amount of enzyme (µg)}}{
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*Adjusted for Substrate Blank
**Using the extinction coefficient 13260 M⁻¹cm⁻¹
***Using the path correction 0.320 cm

Final Assay Conditions: Per Well:
- rhFactor D: 0.25 µg
- DTNB: 100 µM
- 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.

BACKGROUND

Complement Factor D is a serine protease that catalyzes the initial proteolytic step in the alternative pathway of complement. Expressed in adipose tissue at high levels, factor D is also known as adipsin (1). It is an exceptionally specific protease and the only known protein substrate is factor B in complex with C3 (2). Factor D protease activity is regulated by reversible conformational changes, which differs from the majority of serine proteases whose regulation involves either activation by processing of the zymogens or inactivation by binding of the inhibitors. Compared to its physiologically important proteolytic activity, factor D has much lower activity toward synthetic peptide substrates. However, thioester substrates have been routinely used for assessing factor D activity (3).

References: