Recombinant Mouse Thrombomodulin/BDCA-3

Catalog Number: 3894-PA

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse Thrombomodulin/BDCA-3 protein Leu17-Ser517, with a C-terminal 6-His tag Accession # P15306
N-terminal Sequence Analysis	Leu17 & Lys22
Predicted Molecular Mass	55 kDa

SPECIFICATIONS

SDS-PAGE	90-100 kDa, reducing conditions
Activity	Measured by its ability to mediate thrombin activation of protein C.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 0.05 M Tris, 0.01 M $CaCl_2$, 0.15 M NaCl, 0.05 % Brij-35, pH 7.5 (TCNB)
- Recombinant Human Coagulation Factor II/Thrombin (rhThrombin) (Catalog # 1473-SE)
- Recombinant Mouse Thrombomodulin/BDCA-3 (rmTHBD) (Catalog # 3894-PA)
- Recombinant Human Coagulation Factor XIV/Protein C (rhPROC) (Catalog # 3349-SE)
- Recombinant Human Serpin C1 (rhSerpin C1) (Catalog # 1267-PI)
- Heparin (Sigma-Aldrich, Catalog # H3393), 20 mg/mL in deionized water
- Assay Buffer: 0.05 M Tris, 0.1 M NaCl, 0.01% Brij-35, pH 8.5
- Substrate: BOC-Val-Pro-Arg-AMC (Catalog # ES011)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhThrombin to 4 µg/mL in Activation Buffer.
2. Dilute rmTHBD to 1.2 µg/mL in Activation Buffer.
3. Dilute rhPROC to 40 µg/mL in Activation Buffer.
4. Combine 25 µL of the diluted rhPROC, 12.5 µL of the diluted rhThrombin, and 12.5 µL of the diluted rmTHBD in a microfuge tube. Incubate for one hour at 37 °C to activate rhPROC. As a control combine 25 µL of the diluted rhPROC, 12.5 µL of the diluted rhThrombin, and 12.5 µL of Activation Buffer in a microfuge tube.
5. Dilute rhSerpin C1 to 20 µg/mL with 2.7 µg/mL heparin in Activation Buffer.
6. Add and mix 50 µL of the diluted rhSerpin C1/heparin solution to the reaction vials. Incubate at room temperature for 30 minutes to stop rhThrombin activity.
7. Dilute the reaction mixture 1:5 in Assay Buffer.
8. Dilute Substrate to 200 µM in Assay Buffer.
9. Load in a black well plate (F16 Black Maxisorp Plate, Nunc Catalog # 475515) 50 µL of 2 µg/mL of rhPROC (100 ng/well), and start the reaction by adding 50 µL of 200 µM Substrate.
10. Read (top read) immediately in kinetic mode for 5 minutes at excitation and emission wavelengths of 380 nm and 460 nm, respectively.
11. 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 control containing no rmTHBD

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (Sigma-Aldrich, Catalog # A9891).

Final Assay Conditions

Per Well:

- rhPROC: 0.1 µg
- rmTHBD: 1.5 ng
- 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.

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BACKGROUND

Encoded by the THBD gene, Thrombomodulin is also known as CD141 antigen and blood dendritic cell antigen 3 (BDCA-3). The deduced amino acid sequence of mouse THBD predicts a signal peptide (aa 1 to 16) and a mature chain (aa 17 to 577) that consists of the following domains: C-type lectin (aa 31 to 167), EGF-like (aa 240 to 280, aa 283 to 323, aa 324 to 362, aa 364 to 404, aa 405 to 439, and aa 440 to 480), transmembrane (aa 518 to 541) and cytoplasmic (aa 542 to 577) (1). The R&D Systems recombinant mouse THBD consists of aa 17 to 517, corresponding to the extracellular portion of the type I membrane protein.

Predominantly synthesized by vascular endothelial cells, THBD inhibits coagulation and fibrinolysis (2-4). It functions as a cell surface receptor and an essential cofactor for active thrombin, which in turn activates protein C and thrombin-activatable fibrinolysis inhibitor (TAFI), also known as carboxypeptidase B2 (CPB2). Activated protein C (APC), facilitated by protein S, degrades coagulation factors Va and VIIIa, which are required for thrombin activation. Activated CPB2 cleaves basic C-terminal amino acid residues of its substrates, including fibrin, preventing the conversion of plasminogen to plasmin. In addition, THBD gene polymorphisims are associated with human disease and THBD plays a role in thrombosis, stroke, arteriosclerosis, and cancer (5). For example, increased serum levels of THBD, due to protease cleavage, have been associated with smoking, cardiac surgery, atherosclerosis, liver cirrhosis, diabetes mellitus, cerebral and myocardial infarction, and multiple sclerosis (6).

References:

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