Recombinant Human F13A1 His-tag

Catalog Number: 10179-F1

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human F13A1 protein Ser2-Met732 Accession # NP_000120.2 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	No results obtained, Met predicted. Protein identity confirmed by MS analysis of tryptic fragments.
Predicted Molecular Mass	84 kDa

SPECIFICATIONS

SDS-PAGE	76-88 kDa, under reducing conditions
Activity	Measured by its ability to release DNP from Abz-NE(CAD-DNP)EQVSPLTLLK-OH. The specific activity is >13.0 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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, EDTA, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5 (TCNB)
- Recombinant Human F13A1 (rhF13A1) (Catalog # 10179-F1)
- Recombinant Human Coagulation Factor II/Thrombin (Catalog # 1473-SE)
- Substrate: Abz-NE(CAD-DNP)EQVSPLTLLK-OH (Zedira, Catalog # A101), 5 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Florescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Activate rhF13A1 at 100 µg/mL with 40 µg/mL rhThrombin in Assay Buffer for 30 minutes at 37 °C.
2. Dilute activated rhF13A1 to 20 µg/mL in Assay Buffer.
3. Dilute Substrate to 100 µM in Assay Buffer.
4. Load in plate 50 µL of 20 µg/mL activated rhF13-A1, and start the reaction by adding 50 µL of 100 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 100 µM Substrate.
5. Read at excitation and emission wavelengths at 313 nm and 418 nm, respectively, in kinetic mode for 5 minutes.
6. 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 Abz-Gly-OH (Bachem, Catalog # E-2920)

Final Assay Conditions

Per Well:

- rhF13A1: 1 µg
- Substrate: 50 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

DATA

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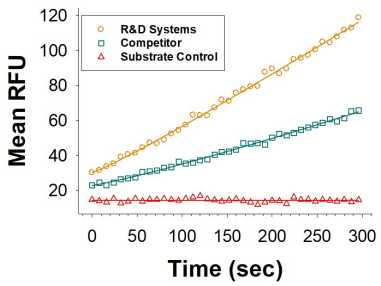
Recombinant Human F13A1 His-tag

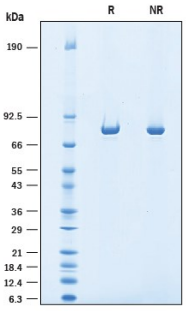
Catalog Number: 10179-F1

Enzyme Activity



Recombinant Human F13A1 His-tag (Catalog # 10179-F1) is measured by its ability to release DNP from Abz-NE(CAD-DNP)EQVSPLTLLK-OH. The activity (orange) is approximately 2-fold higher than the competitor's F13A1 (green).

SDS-PAGE



2 µg/lane of Recombinant Human F13A1 His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® blue staining, showing a band at ~82 kDa under reducing conditions.

BACKGROUND

Coagulation Factor XIIIa1 (F13a1) is a member of the transglutaminase family which includes F13A1 and TGM1-7 (1). F13 in the plasma is a tetrameric complex composed of two alpha (A) and two beta (B) chains where the A subunit is a transglutaminase zymogen and the B subunit is an inhibitory glycoprotein with no enzymatic function (2). Activation by thrombin and calcium ions results in the formation of the catalytically active transglutaminase F13a composed of an alpha chain homodimer capable of forming gamma-glutamyl-epsilon-lysine cross-links. The 83 kDa F13a monomer has an N-terminal activation peptide and a beta sandwich preceding the catalytic core with catalytic triad active site and two C-terminal beta barrels (3). The active homodimer is intracellular in platelets, megakaryocytes, monocytes and macrophages. The primary physiological outcome of the catalytic activity of F13a is cross-linking of fibrin and anti-plasmin to stabilize the fibrin clot (4,5). However, in addition to cross-linking fibrin, F13a is capable of cross-linking many substrates involved in complement activation, coagulation, inflammatory and immune responses and extracellular matrix organization (6). Cross-linking of key substrates by F13a has been directly shown to play a role in in atherosclerosis (7), wound healing (8), angiogenesis (9,10), maintaining pregnancy (11), ECM deposition, osteoblast differentiation and bone remodeling (12), and immune defense (13). F13A has also been detected as a marker in acute promyelocytic leukemia (APL)(14) and expression is considered of value for diagnosis and prognosis for leukemia-associated immunophenotype. Congenital deficiency results in bleeding manifestations including intercranial hemorrhage (15), poor wound healing (17), and spontaneous abortions (17) that can be treated with F13 (18).

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