

DESCRIPTION

Source *Spodoptera frugiperda*, Sf 21 (baculovirus)-derived human Fibroblast Activation Protein alpha/FAP protein
Leu26-Asp760, with an N-terminal 6-His tag
Accession # Q12884

N-terminal Sequence His

Analysis

Predicted Molecular Mass 86 kDa

SPECIFICATIONS

SDS-PAGE 85 kDa, reducing conditions

Activity Measured by its ability to convert the substrate benzyloxycarbonyl-Gly-Pro-7-amido-4-methylcoumarin (Z-GP-AMC) to Z-Gly-Pro and 7-amino-4-methylcoumarin (AMC).
The specific activity is >1800 pmol/min/μg, as measured under the described 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 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 1 M NaCl, 1 mg/mL BSA, pH 7.5
- Recombinant Human Fibroblast Activation Protein α/FAP (rhFAP) (Catalog # 3715-SE)
- Substrate: Z-Gly-Pro-AMC (Bachem, Catalog # I-1145), 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 rhFAP to 0.2 μg/mL in Assay Buffer.
2. Dilute Substrate to 100 μM in Assay Buffer.
3. Load 50 μL of 0.2 μg/mL of rhFAP into a plate, and start the reaction by adding 50 μL of 100 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted } V_{\max}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (μg)}}$$

*Adjusted for Substrate Blank

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

Final Assay Conditions

- Per Well:
- rhFAP: 0.010 μg
 - Substrate: 50 μM

PREPARATION AND STORAGE

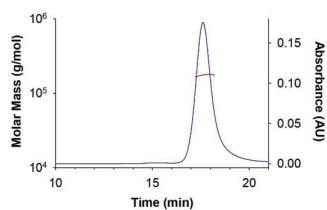
Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

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

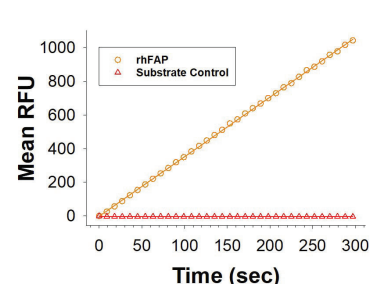
SEC-MALS



SEC-MALS Data	Result
Retention Time	17.2 - 18.2 min
MW - Predicted (Monomer)	86.0 kDa
MW - MALS	176.3 kDa
Polydispersity	1.000
System Suitability	Pass
SEC Monomer	86.4 ± 3.32 kDa

Recombinant Human Fibroblast Activation Protein α /FAP Protein SEC-MALS.
Recombinant Human FAP/His (Catalog # 3715-SE) has a molecular weight (MW) of 176.3 kDa as analyzed by SEC-MALS, suggesting that this protein is a homodimer. MW may differ from predicted MW due to post-translational modifications (PTMs) present (i.e. Glycosylation).

Enzyme Activity



Recombinant Human FAP Protein Enzyme Activity
Recombinant Human FAP His-tag (Catalog # 3715-SE) is measured by its ability to convert the substrate benzyloxycarbonyl-Gly-Pro-7-amido-4-methylcoumarin (Z-GP-AMC) to Z-Gly-Pro and 7-amino-4-methylcoumarin (AMC).

BACKGROUND

FAP (also known as seprase) is a 95 kDa Type II transmembrane serine protease that is structurally related to dipeptidyl peptidase IV (DPPIV/CD26) (1, 2). Within the extracellular domain (ECD), human FAP shares 90% amino acid (aa) sequence identity with mouse and rat FAP (3, 4). Alternative splicing of human FAP generates a truncated isoform that consists of the C-terminal 239 residues of the ECD. A soluble and enzymatically active form of FAP known as antiplasmin-cleaving enzyme (APCE) circulates in human plasma (4). FAP is expressed on reactive stromal fibroblasts in tumor tissue and wound healing and on synovocytes in rheumatoid arthritis (1, 6-8). It exhibits dipeptidyl peptidase activity with substrate specificity similar to DPPIV, which is specific for N-terminal Xaa-Pro sequences (6, 9). FAP is also an endopeptidase that can degrade Gelatin, Collagens I and IV, Fibronectin, and Laminin (1, 6, 9) as well as several peptide hormones (e.g. Neuropeptide Y, Brain Natriuretic Peptide, Substance P, Peptide YY, and Incretins) (10). The enzymatic activity is dependent on FAP association with DPPIV on the cell surface (6, 9, 11, 12). The matrix-degrading activity of FAP contributes to tumor cell migration and invasion (11-14). In addition, FAP can enhance tumor cell growth by limiting the development of anti-tumor immunity (15).

References:

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