Recombinant Mouse Fibroblast Activation Protein α/FAP

Catalog Number: 8647-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived mouse Fibroblast Activation Protein alpha/FAP protein Leu26-Asp761 Accession # P97321
N-terminal Sequence Analysis	Leu26 & Val31
Predicted Molecular Mass	85 kDa

SPECIFICATIONS

SDS-PAGE	78-90 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 >2000 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 Mouse Fibroblast Activation Protein α/FAP (rmFAP) (Catalog # 8647-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 rmFAP 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 rmFAP 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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rmFAP: 0.010 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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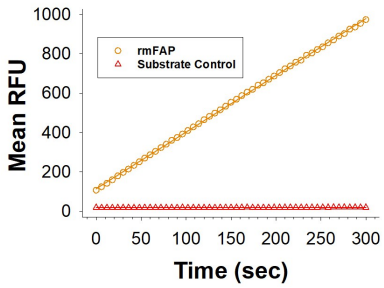
Enzyme Activity



Recombinant Mouse FAP Protein Enzyme Activity
Recombinant Mouse FAP (Catalog # 8647-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, mouse FAP shares 90% and 97% amino acid (aa) sequence identity with human and rat FAP, respectively (3, 4). Alternative splicing of mouse FAP generates isoforms with a 33 aa or 5 aa deletion in the extracellular juxtamembrane region (3). FAP is expressed on reactive stromal fibroblasts in tumor tissue and wound healing and on synoviocytes in rheumatoid arthritis (1, 5-7). It exhibits dipeptidyl peptidase activity with substrate specificity similar to DPPIV, which is specific for N-terminal Xaa-Pro sequences (5, 8). FAP is also an endopeptidase that can degrade Gelatin, Collagens I and IV, Fibronectin, and Laminin (1, 5, 8) as well as several peptide hormones (*e.g.* Neuropeptide Y, Brain Natriuretic Peptide, Substance P, Peptide YY, and Incretins) (9). The enzymatic activity is dependent on FAP association with DPPIV on the cell surface (5, 8, 10, 11). The matrix-dedgrading activity of FAP contributes to tumor cell migration and invasion (10-13). In addition, FAP can enhance tumor cell growth by limiting the development of anti-tumor immunity (14).

References:

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