Recombinant Cynomolgus Monkey Fibroblast Activation Protein α/FAP

Catalog Number: 10278-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey Fibroblast Activation Protein alpha/FAP protein Leu26-Asp760 Accession # XP_005573377
N-terminal Sequence Analysis	Leu26
Predicted Molecular Mass	85 kDa

SPECIFICATIONS

SDS-PAGE	87-94 kDa, under 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 >2500 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 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 Cynomolgus Monkey Fibroblast Activation Protein α/FAP (cynoFAP) (Catalog # 10278-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 rcynoFAP to 0.2 µg/mL in Assay Buffer.
2. Dilute Substrate to 100 µM in Assay Buffer.
3. Load in plate 50 µL of 0.2 µg/mL rcynoFAP, 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.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode of 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 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A9891).

Final Assay Conditions

Per Well:

- rcynoFAP: 0.01 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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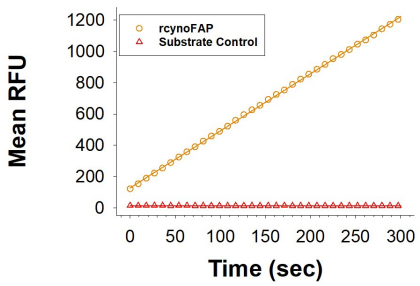
Enzyme Activity



Recombinant Cynomolgus Fibroblast Activation Protein (Catalog # 10278-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-methlycoumarin (AMC).

BACKGROUND

FAP (also known as seprase) is a 95 kDa Type II transmembrane serine protease that forms a homodimer and is structurally related to the dipeptidyl peptidase IV (DPPIV/CD26) family with a short cytoplasmic tail, a single transmembrane domain, and an extracellular domain that contains the active site (1-3). Within the extracellular domain, cynomolgous FAP shares 99.6% and 89.8% amino acid (aa) sequence identity with human and mouse FAP, respectively. It exhibits dipeptidyl peptidase activity with substrate specificity similar to DPPIV, which is specific for N-terminal Xaa-Pro sequences (4, 5). FAP is also an endopeptidase that can degrade Gelatin, Collagens I and IV, Fibronectin, and Laminin (1, 4, 5) as well as several peptide hormones (e.g. Neuropeptide Y, Brain Natriuretic Peptide, Substance P, Peptide YY, and Incretins) (6). FAP is also known to interact with several surface molecules to play roles in cell signaling, cell invasion and migration (3). Although not detectible in normal tissues, FAP is elevated in activated stromal fibroblasts, tumor-associated macrophages, activated hepatic stellate cells and myofibroblasts during pathological conditions that include tissue remodeling such as most types of cancer, wound healing, arthritis, atherosclerosis, and fibrosis (1, 3, 4, 7-9). Targeting FAP with vaccines, antibodies, or pharmacologics impairs tumor progression in several cancer models making it a promising immunotherapy target (9-12).

References:

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