

DESCRIPTION

Source *E. coli*-derived akkermansia muciniphila M60 domain-containing protein
Ala21-Glu506 with an N-terminal Met and C-terminal 6-His tag
Accession # WP_012419679.1

N-terminal Sequence Analysis Met & Ala21

Predicted Molecular Mass 56 kDa

SPECIFICATIONS

SDS-PAGE 52-57 kDa, under reducing conditions

Activity Measured by its ability to digest MUC16.
>90% of MUC16 is digested by rAm M60 peptidase, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >90%, 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.5
- Recombinant A. Muciniphila M60 domain-containing peptidase His-tag (rAm M60 peptidase) (Catalog # 11479-M6)
- Substrate: Recombinant Human CA125/MUC16 Protein (rhMUC16) (Catalog # 5609-MU)
- 4-20% SDS-PAGE Gel Gel loading dye
- Gel Imager/Densitometer

Assay

1. Dilute rAm M60 peptidase to 25 µg/mL with Assay Buffer.
2. Dilute rhMUC16 to 100 µg/mL with Assay Buffer.
3. Combine 10 µL of rAm M60 peptidase and 10 µL of rhMUC16 in a tube.
4. Create a negative control by adding 10 µL of rhMUC16 and 10 µL of Assay Buffer.
5. Incubate reactions and controls at 37 °C for 24 hours.
6. Add gel loading dye to all tubes.
7. Load the total reaction volume on a 4-20% SDS-PAGE gel and run down 80% of the gel, minimally.
8. Stain the gel and acquire image.
9. Use densitometry to calculate the percent digestion of rhMUC16 by rAm M60 peptidase.

Final Assay Conditions Per Reaction:

- rAm M60 peptidase: 0.25 µg
- rhMUC16: 1 µg

PREPARATION AND STORAGE

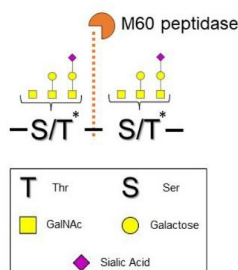
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

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.

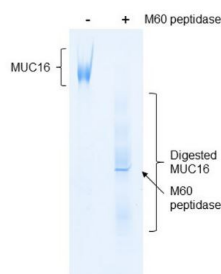
DATA

Enzyme Activity



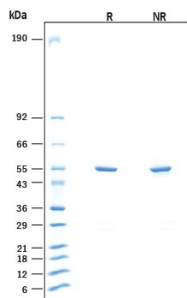
Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein Enzyme Activity Diagram. Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein (Cat # 11479-M6) cleaves glycoproteins N-terminally to glycosylated Ser/Thr residues that are near but not next to the cleavage residue. Specificity requires two adjacent truncated O-glycans and M60 peptidase cleaves between the two residues. * represents the residue for which M60 peptidase has recognition. Sialylated glycan substrates in diagram are less preferred.

Enzyme Activity



Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein Enzyme Activity Assay. Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein (Cat # 11479-M6) is measured by its ability to digest Recombinant Human CA125/MUC16 Protein, CF (Catalog # 5609-MU) and detected via SDS-PAGE gel.

SDS-PAGE



Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant A. Muciniphila M60 domain-containing peptidase His-tag Protein (Catalog # 11479-M6) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 52-57 kDa, under reducing conditions.

BACKGROUND

Recombinant A. Muciniphila M60 domain-containing peptidase (Am M60 peptidase), also referred to as AM0627, is a zinc-dependent mucin-targeting protease from Akkermansia muciniphila, a gut symbiont found within the human and mouse gut mucus layer. Am M60 peptidase is classified as an enzyme within the large M60-like family of metalloproteases containing gluzincin motifs from organisms known to inhabit mucosal host environments (1,2). Am M60 peptidase is a secreted, monomeric protein with a signal sequence, an immunoglobulin-like fold domain, and catalytic domain that contains the zinc-binding site within the gluzincin motif (2,3). Am M60 peptidase is capable of cleaving glycopeptides between adjacent O-glycosylated threonine or serine, with a preference towards desialylated substrates (4). Glycopeptides with adjacent non-physiological truncated O-glycan structure that promote tumorigenesis and metastasis, known as tumor-associated carbohydrate antigens, serve as optimal substrates for Am M60 peptidase (3,5). A. muciniphila relies on host-derived mucin as an energy source and leads to production of short chain fatty acids (SCFA) via mucin-degradation byproducts. SCFA regulate inflammation and provide hosts with health benefits for diseases such as inflammatory bowel disease, obesity, autism, and cancer (2, 6-9). Mucin-degrading enzymes such as Am M60 peptidase play a prominent role in host mucin degradation and consequently Am M60 peptidase may be of interest as a therapeutic target, a diagnostic tool to detect and monitor disease progression, and/or useful as a tool to further study mucins and O-glycoproteins (2-4, 10).

References:

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6. Yamada, T. *et al.* (2019) EbioMedicine **48**: 513.
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