

#### DESCRIPTION

**Source** *E. coli*-derived  
Pro2-Pro303, with an N-terminal Met and 6-His tag  
Accession # Q99685

**N-terminal Sequence Analysis** Met

**Predicted Molecular Mass** 34 kDa

#### SPECIFICATIONS

**SDS-PAGE** 34-37 kDa, reducing conditions

**Activity** Measured by its ability to hydrolyze 4-Nitrophenyl butyrate.  
The specific activity is >15,000 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** >90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

**Formulation** Supplied as a 0.2 μm filtered solution in Tris, NaCl, Brij-35 and EDTA. See Certificate of Analysis for details.

#### Activity Assay Protocol

**Materials**

- Assay Buffer: 50 mM Tris, pH 8.0
- Ethanol, Absolute
- Recombinant Human Monoglyceride Lipase (rhMGLL) (Catalog # 7930-MG)
- Substrate: 4-Nitrophenyl butyrate (PNPB) (Sigma, Catalog # N9876), 100 mM stock in Acetone
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute rhMGLL to 0.8 ng/μL in Assay Buffer.
2. Dilute Substrate to 2 mM using 40% ethanol.
3. Load 50 μL of 0.8 ng/μL rhMGLL into the plate, and start the reaction by adding 50 μL of 2 mM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 2 mM Substrate.
4. Read at an absorbance of 410 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

\*Adjusted for Substrate Blank

\*\*Derived using calibration standard 4-Nitrophenol (4-NP) (Sigma, Catalog # 241326).

**Final Assay Conditions** Per Well

- rhMGLL: 0.04 μg
- PNPB: 1 mM

#### 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.

#### BACKGROUND

Monoacylglycerol lipase, also known as MAG lipase and MGLL, is a member of the serine hydrolase superfamily with the typical serine hydrolase catalytic triad (1). It converts monoacylglycerides to free fatty acids and glycerol. An important physiological process performed by MGLL is the breakdown of the endocannabinoid 2-arachidonoylglycerol (2-AG) by hydrolysis to provide the arachidonic acid precursor for pro-inflammatory eicosanoid synthesis (2). MGLL knock-out mice exhibited enhanced learning and improved performance in novel object recognition due to the increased level of 2-AG (3). In Alzheimer's disease, inactivation of MGLL suppressed accumulation of b-amyloid and reduced the expression of b-site amyloid precursor protein cleaving enzyme 1 (BACE1) (4).

#### References:

1. Karlsson, M. *et al.* 1997, J. Biol. Chem. **272**:27218.
2. Nomura, D. K. *et al.* 2011, Science **334**:809.
3. Pan, B. *et al.* 2011, J. Neurosci. **31**:13420.
4. Chen, R. *et al.* 2012, Cell Rep. **2**:1329.

#### PRODUCT SPECIFIC NOTICES

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