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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
M287-380G-20	20 µg
M287-380G-50	50 µg

MGRN1, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M287-380G

Lot # D2592-8

Product Description

Recombinant human MGRN1 (2-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_015246.

Gene Aliases

CD41B, GP2B, ITGA2B, KIAA0544, RNF156

Formulation

Recombinant proteins stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

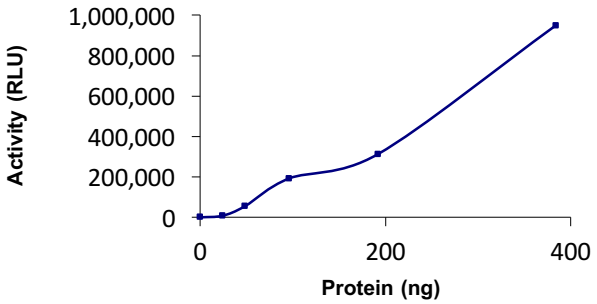
Scientific Background

MGRN1 or mahogunin ring finger 1, E3 ubiquitin protein ligase is a C3HC4 RING-containing protein with E3 ubiquitin ligase activity in vitro (1). MGRN1 gene encodes 4 mRNA isoforms that contain a central C3HC4 RING domain as the only recognizable protein motif and regions flanking the RING domain and the N-terminal region of the protein are also conserved between vertebrate and invertebrate genome and MGRN1 is highly expressed in brain (2). MGRN1 has been detected in the adrenal cortex in kidney and its role may relate to the trafficking and/or degradation of the melanocortin 2 receptor.

References

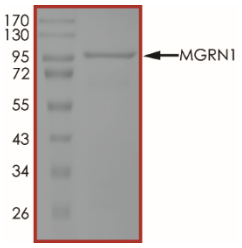
1. Phan, L. K.et.al: The mouse mahoganoid coat color mutation disrupts a novel C3HC4 RING domain protein. J. Clin. Invest. 110: 1449-1459, 2002.
2. He, L. et.al: Spongiform degeneration in mahoganoid mutant mice. Science 299: 710-712, 2003.

Specific Activity



The specific activity of MGRN1 was determined to be **1.4 nmol /min/mg** as per activity assay protocol.

Purity



The purity of MGRN1 was determined to be **>90%** by densitometry. Approx. MW **96 kDa**.

MGRN1, Active

Recombinant human protein expressed in *Sf9* cells

Catalog #	M287-380G
Specific Activity	1.4 nmol/min/mg
Lot #	*D2592-8*
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes

Active MGRN1 (Catalog #: M287-380G), UBA1 (Catalog #: U201-380G) and UBE2D3 (Catalog #: U215-380H) diluted with Ubiquitination Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active MGRN1 for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM $MgCl_2$, 0.1mg/ml BSA. Add 0.5mM DTT prior to use.

AMP-Glo™ Assay (Promega, Catalog #: V5011)

AMP, 10 mM
Ultra Pure ATP, 10mM
AMP-Glo™ Reagent I
AMP-Glo™ Reagent II
Kinase-Glo™ One Solution

Substrate (Catalog #: U06-54N)

Wild-type ubiquitin protein diluted with Ubiquitination Buffer to appropriate working stock.

Assay Protocol

The MGRN1 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin-like (UBL) protein conjugation is proportional to the amount of generated AMP, and the presence of all components of the UBLs conjugation machinery (UBL, E1, E2 and substrate) is required for maximal activity of the system.

Step 1. Thaw the active MGRN1, UBA1, UBE2D3 and ubiquitin on ice, and all AMP-Glo™ components except AMP-Glo™ Reagent II at room temperature. Keep AMP-Glo™ Reagent II on ice.

Step 2. Prepare the following working solutions with Ubiquitination Buffer:

- 2X Reaction Cocktail: 170ng/μl ubiquitin + 15ng/μl UBA1 + 14ng/μl UBE2D3 + 50μM ATP
- 2X final concentration of Active MGRN1

Step 3. In a half-area white 96-well plate, add the following components to bring the initial reaction volume to 10 μl:

Component 1. 5 μl of 2X Reaction Cocktail

Component 2. 5 μl of 2X Active MGRN1

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of Ubiquitination Buffer.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Seal the plate with a plate seal and incubate at 30°C for 2 hours

Step 5. Equilibrate plate to room temperature. Add 10 μl of AMP-Glo™ Reagent I to all wells, mix by shaking for 1-2 minutes. Incubate the plate at room temperature for 60 minutes.

Step 6. Prepare AMP Detection Solution by adding AMP-Glo™ Reagent II to Kinase-Glo™ One Solution at a 1:100 volume ratio. Add 20 μl of the Detection Solution to all wells. Mix for 1-2 minutes and incubate at room temperature for 30 minutes

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 8. Using the AMP standard curve, determine the concentration of AMP produced (μM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine AMP amount from RLUs, see AMP-Glo™ Assay protocol at Promega's website: www.promega.com/protocols

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AMP](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$

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