

TRIM37, Active

Full-length human recombinant protein expressed in Sf9 cells

Catalog # T292-380G

Lot # V2408-19

Product Description

Recombinant full-length human TRIM37 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The TRIM37 gene accession number is [BC036012](#).

Gene Aliases

MUL; POB1; TEF3

Formulation

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

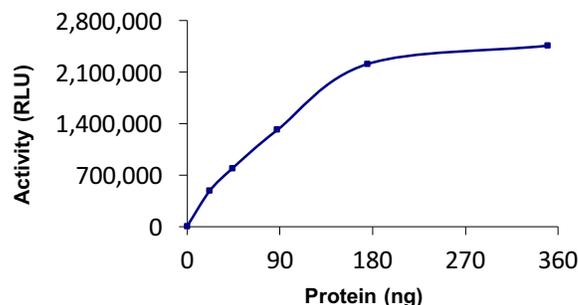
Scientific Background

TRIM37 or tripartite motif containing 37 is a member of the tripartite motif (TRIM) family whose members are involved in diverse cellular functions such as developmental patterning and oncogenesis. The TRIM motif includes zinc-binding domains, a RING finger region, a B-box motif and a coiled-coil domain. The RING finger and B-box domains chelate zinc and might be involved in protein-protein and/or protein-nucleic acid interactions. Mutations in TRIM37 are associated with mulibrey (muscle-liver-brain-eye) nanism, an autosomal recessive disorder that involves several tissues of mesodermal origin.

References

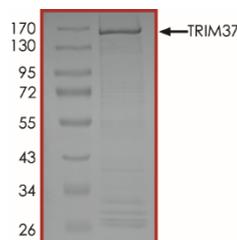
1. Avela, K. et al: Gene encoding a new RING-B-box-coiled-coil protein is mutated in mulibrey nanism. *Nature Genet.* 25: 298-301, 2000
2. Kallijarvi, J. et al: The TRIM37 gene encodes a peroxisomal RING-B-box-coiled-coil protein: classification of mulibrey nanism as a new peroxisomal disorder. *Am. J. Hum. Genet.* 70: 1215-1228, 2002

Specific Activity



The specific activity of TRIM37 was determined to be **16 nmol /min/mg** as per activity assay protocol.

Purity



The purity of TRIM37 was determined to be **>80%** by densitometry, approx. MW **140 kDa**.

TRIM37, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog #	T292-380G
Specific Activity	16 nmol/min/mg
Lot #	V2408-19
Purity	>80%
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 TRIM37 (Catalog #: T292-380G), UBA1 (Catalog #: U201-380G) and UBE2D2 (Catalog #: U214-380H) 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 TRIM37 for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM MgCl₂, 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 a working stock of 170ng/μl (20μM).

Assay Protocol

The TRIM37 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin conjugation is proportional to the generated AMP, and the presence of all components of the Ub conjugation machinery (Ub, E1, E2, and E3) is required for maximal activity of the system.

- Step 1.** Thaw the active TRIM37, UBA1, UBE2D2 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:
 - o 2X Reaction Cocktail: 170ng/μl ubiquitin + 15ng/μl UBA1 + 14ng/μl UBE2D2 + 50μM ATP
 - o 2X final concentration of Active TRIM37
- 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 TRIM37

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 37°C for 60 minutes
- 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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