TOM1 [GST-tagged]

Ubiquitin Binding Protein

Alternate Name: Target of myb1

Cat. No. **66-1015-050**
Lot. No. **30149**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

Ubiquitin signals are decoded in cells by at least 200 ubiquitin binding proteins, which interact with different types of polyubiquitin chains and ubiquitin-like modifiers. These interactions induce conformational changes that allow these proteins to transmit the ubiquitin signal to effector proteins (Dikic *et al.*, 2009). Cloning of the human Target Of Myb1 (TOM1) was first described by Seroussi *et al.* (1999). Human TOM1 shares 76% amino acid sequence identity with chicken TOM1 and 89% identity with mouse TOM1 (Seroussi, *et al.*, 1999). The N-terminal domain of human TOM1 shares sequence similarity to the N-terminal domains of human Signal Transducing Adaptor Molecule (STAM) and Human Growth factor-regulated tyrosine kinase Substrate (HGS) (Seroussi, *et al.*, 1999). TOM1 links polyubiquitin chains to Clathrin (Yamakami, *et al.*, 2003). TOM1 has been shown to bind to human Toll-interacting protein (TOLLIP) via its GAT domain, TOM1 also interacts with Clathrin and when TOM1 and TOLLIP are co-expressed Clathrin is recruited to the endosome suggesting that they may modulate endosomal function (Katoh, *et al.*, 2006). TOM1 directly associates with TOLLIP to form a complex, in which both TOM1 and TOLLIP are capable of directly binding polyubiquitin chains. It is thought that TOM1 is involved in the intracellular sorting of ubiquitylated proteins, analysis of the crystal

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~80.7 kDa

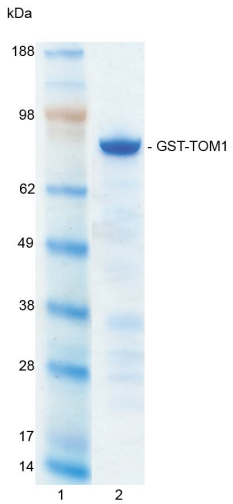
Purity: >85% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

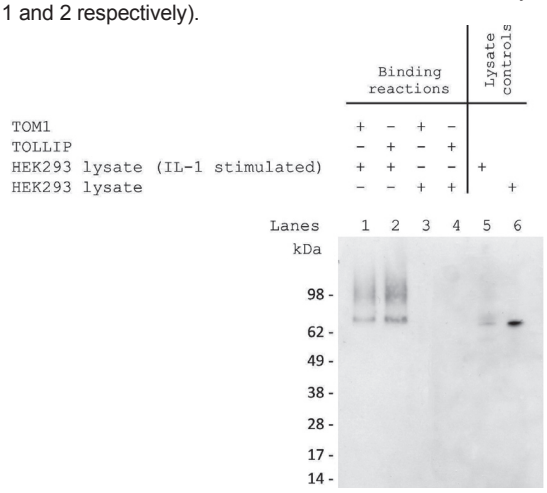
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-TOM1



Protein Identification:
Confirmed by mass spectrometry.

Ubiquitin Binding Domain Activity: The ubiquitin chain binding activity of GST-TOM1 and GST-TOLLIP (Cat# 66-1016-050) were validated through their ability to capture poly ubiquitylated IRAK1 from a lysate preparation derived from IL-1 stimulated HEK293 cells. GST-TOM1 and GST-TOLLIP were pre-incubated with Glutathione Sepharose 4B for 20 minutes at 4°C followed by incubation for 2 hours at 4°C with 2mg IL-1 stimulated HEK293 cell lysate. The binding reaction was then centrifuged and the pellet analysed by SDS-PAGE/Western blotting (Lanes 1 and 2). These samples were compared alongside GST-TOM1 and GST-TOLLIP binding reactions performed with lysates derived from non-stimulated HEK293 cells (Lanes 3 and 4). Ubiquitylated IRAK1 was identified by Western Blotting using an anti-IRAK1 antibody and such species were observed only in the pellet sample derived from a binding reaction containing wild-type GST-TOM1 or GST-TOLLIP and IL-1 stimulated HEK293 cell lysate (Lanes 1 and 2 respectively).



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Lot-specific COA version tracker: v1.0.0

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structure of the TOM1-GAT domain with ubiquitin has revealed the presence of two ubiquitin binding domains (Akutsu, *et al.*, 2005).

References:

Akutsu M, Kawasaki M, Katoh Y, Shiba T, Yamaguchi Y, Kato R, Kato K, Nakayama K, Wakatsuki S (2005) Structural basis for recognition of ubiquitinated cargo by Tom1-GAT domain, *FEBS Lett* **579**, 5385-5391.

Katoh Y, Imakagura H, Futatsumori M, Nakayama K (2006) Recruitment of clathrin onto endosomes by the Tom1-Tollip complex, *Biochem Biophys Res Comm* **341**, 143-149.

Seroussi E, Kedra D, Kost-Alimova M, Sandberg-Nordqvist AC, Fransson I, Jacobs JF, Fu Y, Pan HQ, Roe BA, Imreh S, Dumanski JP (1999) TOM1 genes map to human chromosome 22q13.1 and mouse chromosome 8C1 and encode proteins similar to the endosomal proteins HGS and STAM, *Genomics* **57**, 380-388.

Yamakami M, Yoshimori T. and Yokosawa H (2003) Tom1, a VHS domain-containing protein, interacts with tollip, ubiquitin, and clathrin, *J Biol Chem* **278**, 52865-52872.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYM
DPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEV
LFQGPLGS***M***DFLLGNPFSSPVGQRIEKA
TDGSLQSEDWALNMEICDIINETEEG
PKDALRAVKKRIVGNKNFHEVMLALTV
LETCVKNCGHRFHVLVASQDFVESVLVRTIL
PKNNPPTIVHDKVLNLIQSWADAFRSSPDLT
GVVTIYEDLRRKGLEFPMTDLDMLSPIHT
PQRTVFNSETQSGQDSVGTDSSQQEDS
GQHAAPLPAPPILSGDTPIAPTPEQIGKL
RSELEMVSGNVRVMSEMLTELVPTQAEPA
DLELLQELNRTCRAMQQRVLELIPQIANEQL
TEELLIVNDNLNNVFLRHERFERFRTGQTT
KAPSEAEPAADLIDMGPDPAATGNLSSQLAG
MNLGSSSVRAGLQSLEASGRLEDEFDMFAL
TRGSSLADQRKEVKYEAPQATDGLAGALDAR
QQSTGAIPVTQACLMEDIEQWLSTDVGN
DAEEPKGVTSEGKFDKFLEERAKAADRLPN
LSSPSAEGPPGPPSGPAPRKKTQEKDDDML
FAL

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
TOM1 (regular text): Start ***bold italics*** (amino acid residues 1-493)
Accession number: NP_001129204.1

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