TOLLIP [GST-tagged]

Ubiquitin Binding Protein

Alternate Name: IL-1RAcPIP

Cat. No. **66-1016-050**
Lot. No. **30150**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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 Toll-interacting protein (TOLLIP) was first described by Burns *et al.* (2000). TOLLIP has an N-Terminal TOM1 binding domain (TBD) that mediates protein-protein interactions, a C2 domain that targets TOLLIP to the endosome and a C-terminal CUE domain that binds mono-ubiquitin (Lo *et al.*, 2009). Recent studies have proposed that Interleukin 1B (IL-1B) stimulation of HEK293 cells induces aggregation of Interleukin 1 Receptors (IL-1Rs) and recruitment of MYD88 followed by the TOLLIP/IL-1 receptor-associated kinase 1 (IRAK1) complex. Phosphorylation of IRAK by MYD88 then leads to the dissociation of TOLLIP from IRAK, which can then transmit the IL1-induced signals (Burns *et al.*, 2000). PTEN-induced putative kinase 1 (PINK1) specifically binds to two components of the IL-1 mediated signalling cascade, TOLLIP and IRAK1. Association of PINK1 with TOLLIP facilitates the dissociation of TOLLIP from IRAK1, which in turn facilitates the assembly of the IRAK1/TNF receptor-associated factor 6 (TRAF6) complex and also the Lys

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~51.7 kDa

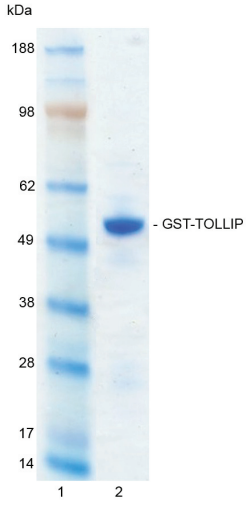
Purity: >90% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

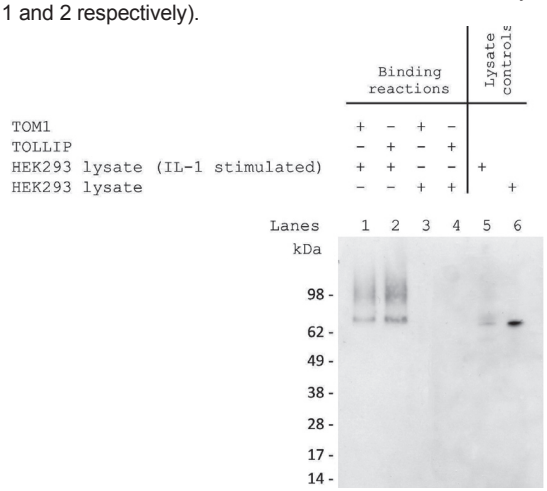
Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.

Ubiquitin Binding Domain Activity: The ubiquitin chain binding activity of GST-TOM1 (Cat# 66-1015-050) and GST-TOLLIP 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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www.ubiquigent.com
Dundee, Scotland, UK

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International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

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63 linked polyubiquitylation of IRAK1 (Lee *et al.*, 2012). Human Target of Myb1 (TOM1) has been shown to bind to 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 and it has been proposed that TOM1 links polyubiquitin chains to Clathrin (Yamakami *et al.*, 2003).

References:

Burns K, Clatworthy J, Martin L, Martinon F, Plumpton C, Maschera B, *et al.* (2000) Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. *Nat Cell Biol* **2**, 346-351.

Dikic I, Wakatsuki S and Walters KJ (2009) Ubiquitin-binding domains - from structures to functions. *Nat Rev Mol Cell Biol* **10**, 659-671.

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

Lee HJ and Chung KC (2012) PINK1 positively regulates IL-1beta-mediated signaling through Tollip and IRAK1 modulation. *J Neuroinflam* **9**, 271.

Lo YL, Beckhouse AG, Boulus SL and Wells CA (2009) Diversification of TOLLIP isoforms in mouse and man. *Mamm Gen* **20**, 305-314.

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:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLY
DALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQ
IDKYLKSSKYIAWPLQGWQATFGGGDHPPKS
DLEVLFQGPLGS***M***ATTVSTQRGPVYIGELPQD
FLRITPTQQQRQVQLDAQAAQQLQYGGAVGT
VGRLNITVVQAKLAKNYGMTRMDPYCRLRLG
YAVYETPTAHNGAKNPRWNKVIHCTVPPGVDS
FYLEIFDERAFSMDDRIAWTHITIPESLRQG
KVEDKWYSLSGRQGDDKEGMINLVMSYALL
PAAMVMPPQPVVLMPTVYQQGVGYVPITGM
PAVCSPGMVPVALPPAAVNAQPRCSEEDLKAI
QDMFPNMDQEVIRSVLEAQRGNKDAAINSLLQM
GEEP

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
TOLLIP (regular text): Start ***bold italics*** (amino acid residues 1-274)
Accession number: NP_061882.2

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Dundee, Scotland, UK

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Lot-specific COA version tracker: v1.0.0