

# 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

## 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

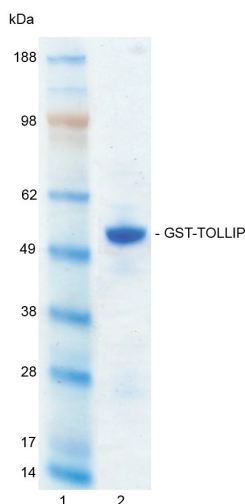
## Quality Assurance

### Purity:

4-12% gradient SDS-PAGE  
InstantBlue™ staining

Lane 1: MW markers

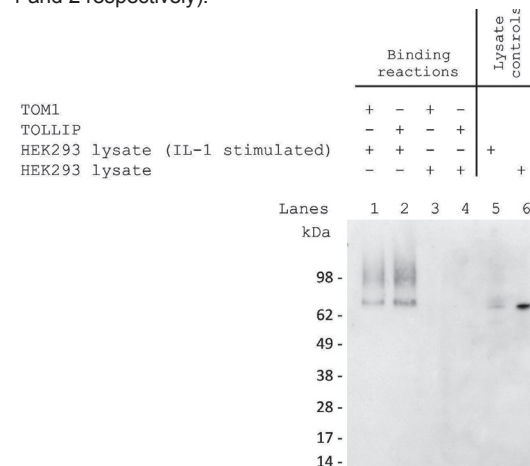
Lane 2: 1 µg GST-TOLLIP



### 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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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 Neuroinflamm* 9, 271.

Lo YL, Beckhouse AG, Boulos 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**  
**LEGAVLDIRYGVSR IAYS KDFETLKVDFL**  
**SKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLY**  
**DALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQ**  
**IDKYLKSSKYIAWPLQGWAATFGGGDHPPKS**  
**DLEVLFGQPLGSMATTVSTQRGPVYIGELPQD**  
FLRITPTQQQRQVQLDAQAAQQLQYGGAVGT  
VGRLNITVVQAKLAKNYGMTRMDPYCRLRLG  
YAVYETPTAHNGAKNPRWNKVIHCTVPPGVDS  
FYLEIFDERAFSMDRIAETHITIPESLRQG  
KVEDKWYSLSGRQGGDDKEGMINLVMSYALL  
PAAMVMPPQPVVLMPTVYQQGVGYVPI TGM  
PAVCSPGMVPVALPPAAVNAQPRCSEEDLKAI  
QDMFPNMDQEVIRSVLEAQRGNKDAAINSLQM  
GEEP

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVLFGQ▼GP)

TOLLIP (regular text): Start **bold italics** (amino acid residues 1-274)

Accession number: NP\_061882.2



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