TRAF6 (mouse; full length), pAb

Alternate Names: TNF receptor associated factor 6; RNF85; MGC:3310

Cat. No.	**68-0022-100**	Quantity:	100 µg
Lot. No.	**30259**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

TRAF (Tumour Necrosis Factor Receptor-associated factor) proteins are ubiquitin E3 ligases. TRAF proteins have a pivotal role in signalling pathways that are involved in the activation of NF-κB by many cell-surface receptors, including the TNFR superfamily, the IL-1 receptor (IL-1R) and Toll-like receptors (TLRs). Cloning of TRAF6 from human 293 cells was first described by Cao *et al.* (1996). TRAF6 is essential for IKK and Jun N-terminal kinase (JNK) activation in the IL-1 and TLR pathways. In these pathways, stimulation of the receptors leads to the recruitment of MyD88, an adaptor protein that further recruits the protein kinases IRAK4 and IRAK1. IRAK1 then binds to TRAF6, which subsequently activates downstream signalling cascades, including those of IKK and JNK (Chen 2005). TRAF6 is a known E3 ligase for Akt (protein kinase B) and is essential for Akt K63-chain ubiquitylation. Akt signalling plays a central role in many biological functions, such as cell proliferation and apoptosis. The human cancer-associated Akt mutant displays an increase in Akt ubiquitylation, in turn contributing to the enhancement of Akt membrane localization and phosphorylation. Therefore, ubiquitylation of Akt by TRAF6 is an important step for oncogenic Akt activation (Yang *et al.* 2009).

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: mouse TRAF6 (residues 1-530)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects TRAF6 at ~60 kDa

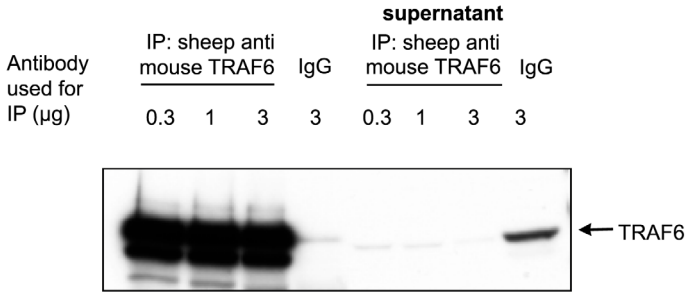
Reactivity: mouse; other species not tested

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Not tested

Immunoprecipitation:
Use 1 µg/mg of cell extract



Immunoprecipitation Assay:
TRAF6 was immunoprecipitated from RAW 264.7 total cell extracts (1 mg) using varying amounts of anti-TRAF6 antibody (Cat# 68-0022-100) or pre-immune serum (IgG). TRAF6 was subsequently detected by Western Blot using a commercially available anti-TRAF6 antibody. In order to show that all TRAF6 was immunoprecipitated from the input cell extract, a Western Blot was carried out using anti-TRAF6 antibody on 25 µg of the cell extract following immunoprecipitation and no TRAF6 could be detected. This demonstrates that 1 µg of the TRAF6 antibody can completely deplete TRAF6 from 1 mg of cell extracts.

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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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Background

Continued from page 1

Antibody Production:
Anti-TRAF6 (mouse) polyclonal antibody was raised in sheep against TRAF6 (residues 1-530 of mouse TRAF6). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-TRAF6 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-TRAF6 (mouse) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV (1996) TRAF6 is a signal transducer for interleukin-1. *Nature* **383**, 443-6.

Chen ZJ (2005) Ubiquitin signalling in the NF-kappaB pathway. *Nat Cell Biol* **7**, 758-65.

Yang WL, Wang J, et al. (2009) The E3 ligase TRAF6 regulates Akt ubiquitination and activation. *Science* **325**, 1134-8.

Application Reference:

Pauls E1, Nanda SK, Smith H, Toth R, Arthur JS, Cohen P (2013) Two phases of inflammatory mediator production defined by the study of IRAK2 and IRAK1 knock-in mice. *J Immunol* **191**, 2717-30.

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Lot-specific COA version tracker: v1.0.0