





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

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Tripartite Motif containing 65 (TRIM65) is a member of the E3 protein ligase family and cloning of the gene was first described by Strausberg et al. (2002). TRIM65, is a 517 amino acid protein that belongs to the TRIM/RING Finger-B Box-Coiled Coil (RBCC) family and is encoded by a gene located on human chromosome 17g25.1 (Jensen et al., 2001; Fornage et al., 2011). The TRIM family of proteins are characterized by a conserved TRIM domain that includes a RING finger domain, three zinc-binding domains, a coiled-coil region and a B box-type zinc Finger. Several of the TRIM proteins are upregulated by type I and type II interferons, which are essential for the development of pathogen-resistance (Nisole, et al., 2005; Ozato et al., 2008; Mc-Nab et al., 2010).

Antibody Production:

Anti-TRIM65 (human) polyclonal antibody was raised in sheep against TRIM65 (residues 1-485

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TRIM65 (human; full length), pAb

Alternate Names: Tripartite Motif-Containing 65, 4732463G12Rik

Cat. No. 68-0024-100 Quantity: 100 µg -20°C Lot. No. 30261 Storage:

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on

shipping

Source: sheep polyclonal antibody

Immunogen: human TRIM65 (residues

1-485) [GST-tagged]

Purification: affinity-purified using

immobilized immunogen

Formulation: phosphate-buffered

Specificity: detects TRIM65 at

~57 kDa

Reactivity: human; other species not

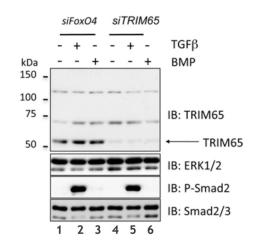
tested.

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

Research Applications and Quality Assurance

Western Immunoblotting: Use 1.0 µg/ml

Immunoprecipitation: Use 5.0 µg/mg of cell extract



Western Blotting Analysis:

By Western blotting TRIM65 was not detected in lysates from cells transfected with siTRIM65 (lanes 4-6) compared to those cells transfected with control siFoxO4 (lanes 1-3) where TRIM65 could be detected when probed with 0.1 µg/ml anti-TRIM65 antibody (Cat# 68-0024-100).



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Lot-specific COA version tracker: v1.0.0







Background

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of human TRIM65). 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-TRIM65 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-TRIM65 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA et al. (2011) Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. Ann Neurol 69, 928-39.

Jensen K, Shiels C, Freemont PS (2001) PML protein isoforms and the RBCC/TRIM motif. Oncogene 20, 7223-7233

McNab FW, Rajsbaum R, Stoye JP, O'Garra A (2010) Tripartite-motif proteins and innate immune regulation. Curr Opin Immunol 23, 46-56.

Nisole S, Stoye JP, Saïb A (2005) TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 3, 799-808

Ozato K, Shin DM, Chang TH, Morse HC (2008) TRIM family proteins and their emerging roles in innate immunity. Nat Rev Immunol 8, 849-860.

Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD (2002) Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. PNAS 99, 16899-903.

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