RNF7 (human; full length), pAb

Alternate Names: RING-box protein 2 isoform 1, Rbx2, Regulator of cullins 2, ROC2, SAG

Cat. No.	**68-0020-100**	Quantity:	100 µg
Lot. No.	**30257**	Storage:	-20˚C

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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

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). Ring Finger protein 7 (RNF7) is a member of the E3 protein ligase family and cloning of the human gene was first described by Ohta *et al.* (1999). RNF7 is a subunit of the Cullin RING (1-5) E3 ubiquitin ligase (CRL) complexes which bind and recruit the ubiquitin E2 conjugating enzymes (Sarikas *et al.*, 2011; Sun *et al.*, 2013). RNF7 silencing has been shown to selectively inhibit cancer cell proliferation, suppress *in vivo* tumour growth, and sensitise radiation-resistant cancer cells to radiation by inducing apoptosis (Jia *et al.*, 2010). Both RBX1 and RNF7 are overexpressed in human lung cancer, however it has been shown that the overexpression of RNF7 is correlated with poor patient prognosis and more advanced disease. RNF7, also known as RBX2/ROC2/SAG (RING-box 2/regulator of cullins 2/sensitive to apoptosis gene), has been demonstrated to be a K*ras*-cooperating oncogene that promotes lung tumourigenesis and it is thought that targeting RNF7-CRL

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human RNF7 (residues 1-113) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects RNF7 at ~12 kDa

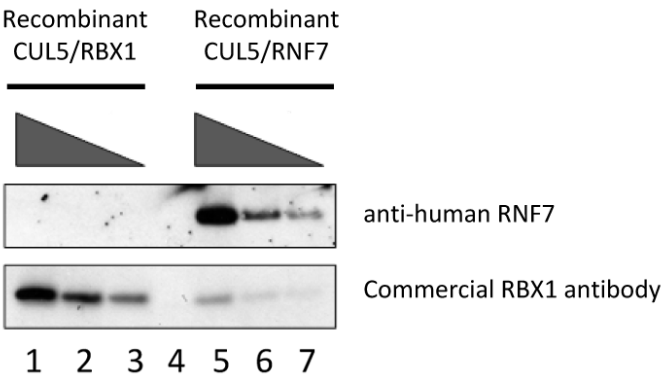
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
Use 1.0 µg/ml

Immunoprecipitation:
Use 20 µg/mg of cell extract



Western Blotting Analysis:
In insect cells infected with baculovirus expressing either CUL5/RBX1 or CUL5/RNF7 RNF7 was detected (lanes 5-7) when probed with the anti-human RNF7 antibody (Cat# 68-0020-100) without cross-reacting with the closely related Rbx1 protein.

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Lot-specific COA version tracker: v1.0.0

RNF7 (human; full length), pAb

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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

E3 ligases may be an effective therapeutic approach for K*ras* driven lung cancers (Li *et al.*, 2014).

Antibody Production:
Anti-RNF7 (human) polyclonal antibody was raised in sheep against RNF7 (residues 1-113 of human RNF7). 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-RNF7 pAbs from the sheep serum using an antigen-agarose column followed by depletion of any anti-GST pAbs using a GST-agarose column. Anti-RNF7 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Jia L, Yang J, Hao X, Zheng M, He H, Xiong X, Xu L, Sun Y (2010) Validation of SAG/RBX2/ROC2 E3 ubiquitin ligase as an anticancer and radiosensitising target. *Clin Cancer Res* **3**, 814-24.

Li H, Tan M, Jia L, Wei D, Zhao Y, Chen G, Xu J, Zhao L, Thomas D, Beer DG, Sun Y (2014) Inactivation of SAG/RBX2 E3 ubiquitin ligase suppresses KrasG12D-driven lung tumorigenesis. *J Clin Invest* **2**, 835-46.

Ohta T, Michel JJ, Schottelius AJ, Xiong Y (1999) ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Molec Cell* **3**, 535-541.

Sarikas A, Hartmann T, Pan ZQ (2011) The cullin protein family. *Genome Biol* **12**, 220.

Sun Y, Li H (2013) Functional characterization of SAG/RBX2/ROC2/RNF7, an antioxidant protein and an E3 ubiquitin ligase. *Protein Cell* **2**, 103-16.

Application Reference:

Kelsall IR, Duda DM, Olszewski JL, Hofmann K, Knebel A, Langevin F, Wood N, Wrightman M, Schulman BA, and Alpi AF (2013) TRIAD1 and HHARI bind to and are activated by distinct neddylated Cullin-RING ligase complexes. *EMBO J* **32**, 2848-60.

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Lot-specific COA version tracker: v1.0.0