Store at -20°C

ROS1 (D4D6®) Rabbit mAb

#3287

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Entrez-Gene ID #6098
UniProt ID #P08922

rev. 09/15/16

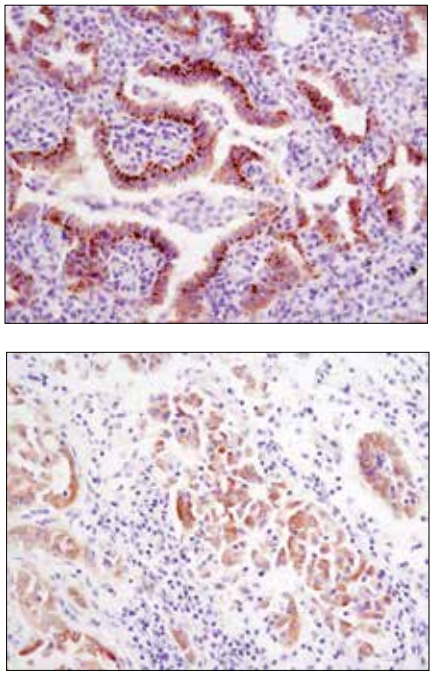
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H	258, 110, 50-80 kDa	Rabbit IgG**

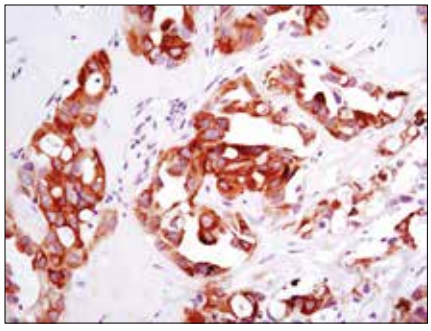
Background: ROS1, an orphan receptor tyrosine kinase of the insulin receptor family, was initially identified as a homolog of v-ros from the UR2 sarcoma virus (1). ROS1 consists of a large extracellular domain that is composed of six fibronectin repeats, a transmembrane domain, and an intracellular kinase domain. While the function of ROS1 is undefined, it has been shown to play an important role in differentiation of epididymal epithelium (2). The first oncogenic fusion of ROS1, FIG-ROS1, was initially identified by research studies in glioblastoma (3), and subsequent studies have found this fusion in cholangiocarcinoma (4), ovarian cancer (5) and non-small cell lung cancer (NSCLC) (6). Investigators have found additional oncogenic ROS1 fusion proteins in NSCLC (at a frequency of ~1.6%), where the ROS1 kinase domain is fused to the amino-terminal region of a number of different proteins, including CD74 and SLC34A2 (6-8). ROS1 fusion proteins activate the SHP-2 phosphatase, PI3K/Akt/mTOR, Erk, and Stat3 pathways (3,4,9).

Specificity/Sensitivity: ROS1 (D4D6®) Rabbit mAb recognizes endogenous levels of total ROS1 protein. This antibody does not cross-react with other related proteins when analyzed by western blot. Please note that staining may be observed in ROS1 rearranged lung carcinomas, macrophages/giant cells, reactive type II pneumocyte hyperplasia, and the epithelium in areas of bronchiolar metaplasia. Staining of unknown specificity has been observed in cholangiocarcinoma, hepatocellular carcinoma, and kidney tissues.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a protein corresponding to residues in the carboxy terminal domain of the human ROS1 protein.



Immunohistochemical analysis of paraffin-embedded human lung carcinomas showing distinct localization using ROS1 (D4D6®) Rabbit mAb.



◀ *Immunohistochemical analysis of paraffin-embedded HCC78 xenograft using ROS1 (D4D6®) Rabbit mAb.*

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:250†
Unmasking buffer:	EDTA
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:400
Flow Cytometry	1:100

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:

(1) Matsushime, H. et al. (1986) *Mol Cell Biol* 6, 3000-4.

(2) Yeung, C.H. et al. (1999) *Biol Reprod* 61, 1062-9.

(3) Charest, A. et al. (2003) *Genes Chromosomes Cancer* 37, 58-71.

(4) Gu, T.L. et al. (2011) *PLoS One* 6, e15640.

(5) Birch, A.H. et al. (2011) *PLoS One* 6, e28250.

(6) Rimkunas, V.M. et al. (2012) *Clin Cancer Res* 18, 4449-57.

(7) Rikova, K. et al. (2007) *Cell* 131, 1190-203.

(8) Stumpfova, M. and Jänne, P.A. (2012) *Clin Cancer Res* 18, 4222-4.

(9) Jun, H.J. et al. (2012) *Cancer Res* 72, 3764-74.

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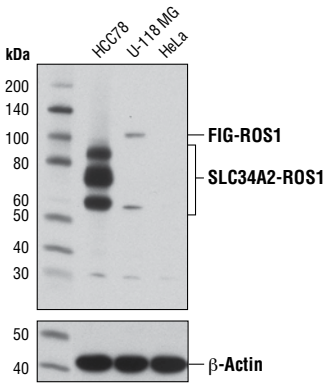
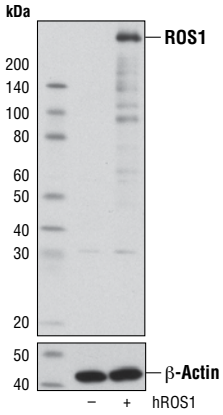
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

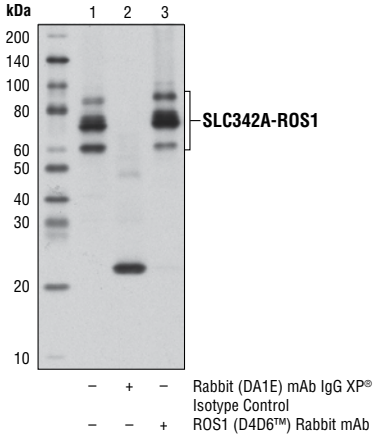
Cell Signaling TECHNOLOGY®



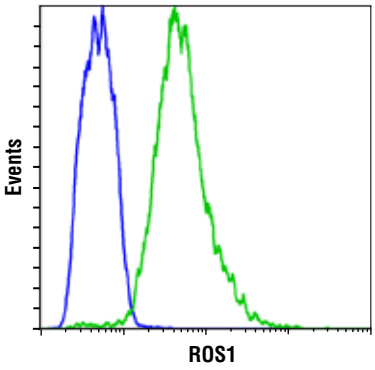
Western blot analysis of extracts from HCC78 (SLC34A2-ROS1), U-118 MG (FIG-ROS1), and HeLa (ROS1 negative) cells using ROS1 (D4D6®) Rabbit mAb (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower). Note: HCC78 cells express the 85, 70, and 59 kDa forms of the SLC34A2-ROS1 fusion protein (7).



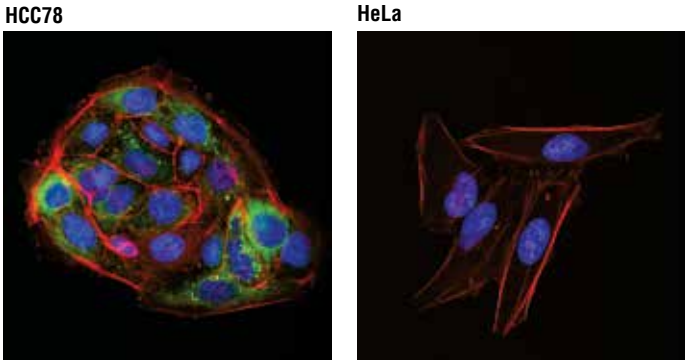
Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with a construct expressing wildtype ROS1 (hROS1; +), using ROS1 (D4D6®) Rabbit mAb (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).



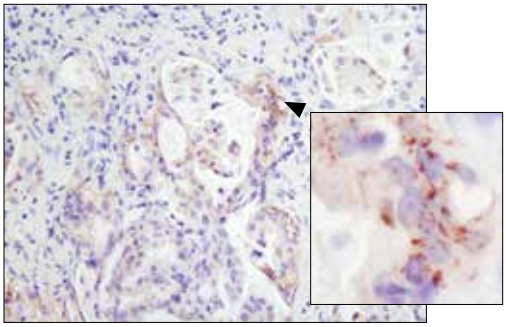
Immunoprecipitation of ROS1 from HCC78 cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or ROS1 (D4D6®) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using ROS1 (D4D6®) Rabbit mAb, followed by Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb #3678 and Anti-mouse IgG, HRP-linked Antibody #7076.



Flow cytometric analysis of HeLa (blue) and HCC78 (green) cells using ROS1 (D4D6®) Rabbit mAb. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



Confocal immunofluorescent analysis of HCC78 (left) or HeLa (right) cells using ROS1 (D4D6®) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using ROS1 (D4D6®) Rabbit mAb. Note: Staining is of FIG-ROS1 fusion (6).