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Datasheet for 200-301-397

SMC1 phospho S957 Antibody

Overview

Description:	Anti-SMC1 pS957 (MOUSE) Monoclonal Antibody - 200-301-397
Item No.:	200-301-397
Size:	100 µg
Applications:	ELISA, IHC, WB, IF, Multiplex
Reactivity:	Human, Mouse
Host Species:	Mouse

Product Details

Background:	Structural maintenance of chromosomes (SMC) proteins play important roles in sister chromatid cohesion, chromosome condensation, sex-chromosome dosage compensation, and DNA recombination and repair (DNA damage). Protein complexes containing heterodimers of the SMC1 and SMC3 proteins have been implicated specifically in both sister chromatid cohesion and DNA recombination. ATM, a protein kinase belonging to the phosphatidylinositol 3-kinase family that regulates cell cycle checkpoints and DNA recombination and repair, phosphorylates SMC1 protein after ionizing irradiation. ATM protein kinase phosphorylates SMC1 on serines 957 and 966 in vitro and in vivo, and expression of an SMC1 protein mutated at these phosphorylation sites abrogates the ionizing irradiation-induced S phase cell cycle checkpoint. Optimal phosphorylation of these sites in SMC1 after ionizing irradiation also requires the presence of the ATM protein kinase substrates NBS1 and BRCA1. These same sites in SMC1 are phosphorylated after treatment with UV irradiation or hydroxyurea in an ATM-independent manner, thus demonstrating that another kinase must be involved in responses to these cellular stresses. Yeast containing hypomorphic mutations in SMC1 and human cells overexpressing SMC1 mutated at both of these phosphorylation sites exhibit decreased survival following ionizing irradiation. These results demonstrate that SMC1 participates in cellular responses to DNA damage and link SMC1 to the ATM protein kinase signal transduction pathway.
Synonyms:	mouse anti-SMC1 pS957 antibody, Structural maintenance of chromosomes protein 1B antibody, SMC1beta protein antibody, SMC1B antibody, SMC1L2 antibody
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	5D11.G5
Format:	IgG1

Target Details

Gene Name:	SMC1B
Reactivity:	Human, Mouse
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This antibody was produced from a synthetic phospho-peptide corresponding to aa 951-962 of human SMC1 by injection into a balb/c mouse.
Purity/Specificity:	This Protein G Purified Mab antibody is directed against human SMC1 and is useful in determining its presence in various assays. This monoclonal anti-SMC1 antibody recognizes the phosphorylated epitope in native and over-expressed proteins found in various tissues and extracts. Minimal reactivity is observed against the non-phosphorylated epitope. Reactivity is observed against human and mouse SMC1. Cross reactivity with SMC1 from other eukaryotic sources has not been tested.
Relevant Links:	• UniProtKB - Q8NDV3 • NCBI - Q8NDV3.2 • GeneID - 27127

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF, Multiplex (Based on references)
Application Note:	Protein G Purified Mab anti-SMC1 was tested by ELISA, immunohistochemistry and western blotting against native protein. The antibody reacts with SMC1 from irradiated human and mouse cells. A 160 kDa band corresponding to phosphorylated human SMC1 is noted in gamma irradiated human and mouse lysates.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:100,000
IF:	2.5 µg/ml
IHC:	2.5 µg/ml
WB:	1:100 - 1:2,000

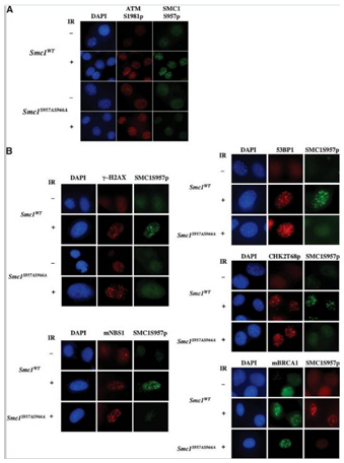
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

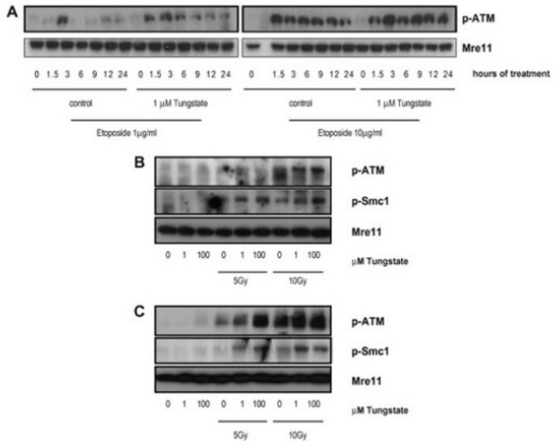
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

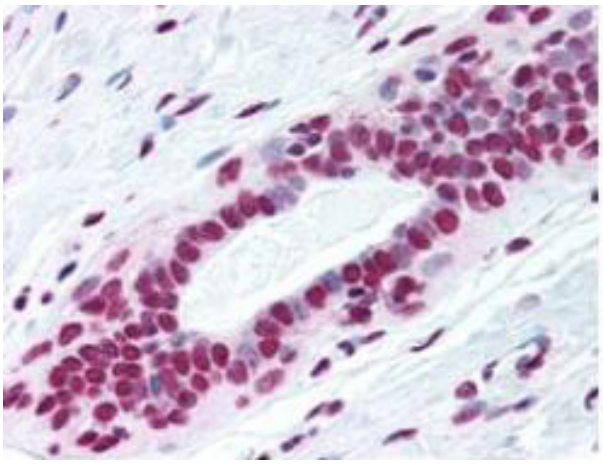
Images



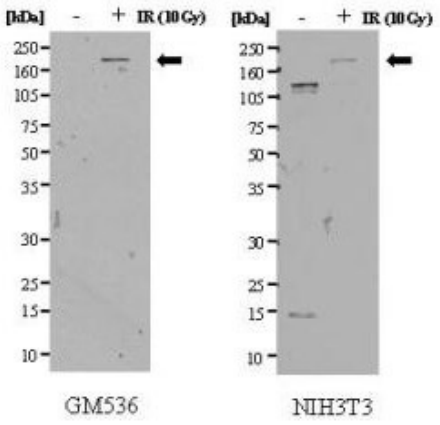
Immunofluorescence Microscopy
SMC1 phosphorylation is not required for IR-induced formation of foci containing phospho-ATM, H2AXγ, NBS1, 53BP1, phosphorylated CHK2, or BRCA1. Wild-type (Smc1WT) or Smc1 phosphorylation mutant knock-in (Smc1S957AS966A) fibroblast cells were fixed with 4% paraformaldehyde 30 min after 0 Gy (-) 10 Gy (+) of ionizing irradiation (IR), then subjected to immunofluorescence microscopy. (A) Staining with antibodies recognizing phosphorylated ATM or SMC1. (B) Staining with antibodies recognizing H2AXγ, NBS1, 53BP1, phosphorylated CHK2, BRCA1, and phosphorylated SMC1. For costaining of phosphorylated SMC1 (red) with BRCA1 (green), rabbit polyclonal anti-Ser957p antibody was used. Fig 6. PMID: 15175241



Western Blot
Tungstate modulates ATM and Smc1 phosporylation kinetics upon DNA damage. (A) Immunodetection of phospho-ATM (p-ATM) in HeLa cells treated with 1 or 10 µg/mL etoposide for the hours indicated ±1 µM tungstate. (B) Immunodetection of p-ATM and phospho-Smc1 (p-Smc1) in HeLa cells irradiated with 5 or 10 Gy and with indicated concentrations of tungstate for 3 h. (C) Immunodetection of p-ATM and phospho-Smc1 (p-Smc1) in wild-type MEFs irradiated with 5 or 10 Gy and with indicated concentrations of tungstate for 3 h. Even loading of the gels was confirmed by immunodetection of Mre11 protein.
Fig 3. PMID: 23587483



Immunohistochemistry
Rockland's Protein G Purified Mab anti-SMC1 pS957 antibody was used at a 2.5 µg/ml to detect nuclear signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate to strong nuclear anti-SMC1 pS957 staining of human breast ductal epithelium. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpan Biosciences, Seattle, WA.



Western Blot
Western blot of gamma irradiated (+ lanes) and mock-irradiated (- lanes) human GM536 lymphoblastoid cell lysate (left panel) and mouse NIH3T3 cell lysate (right panel). Rockland's Protein G Purified Mab anti-SMC1 pS957 detects a 160 kDa band corresponding to phosphorylated SMC1. The antibody does not react with non-phosphorylated SMC1 present in the human control lane. Non specific binding may occur in control lanes of lysates from mouse cell origins. The cell lysates were prepared in a RIPA buffer containing 200 mM NaCl, and 20 µg protein was loaded per lane. A 4-12% Bis-Tris gradient gel (Invitrogen) was used for SDS-PAGE. The membrane was probed with the primary antibody at 10µg/ml for 1 h at 20°C followed by washes and reaction with a 1:1000 dilution of HRP conjugated Dnky-a-Mouse IgG [H&L] (code 610-703-124) for 30 min.

References

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- Kitagawa et al. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. *Genes Dev.* (2004)

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