

#4805 Store at **-20°C**

Phospho-SMC1 (Ser957) (5D11G5) Mouse mAb

100 µl
 (10 Western mini-blot)



Orders ■ 877-616-CELL (2355)
 orders@cellsignaling.com
Support ■ 877-678-TECH (8324)
 info@cellsignaling.com
Web ■ www.cellsignaling.com

rev. 01/22/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

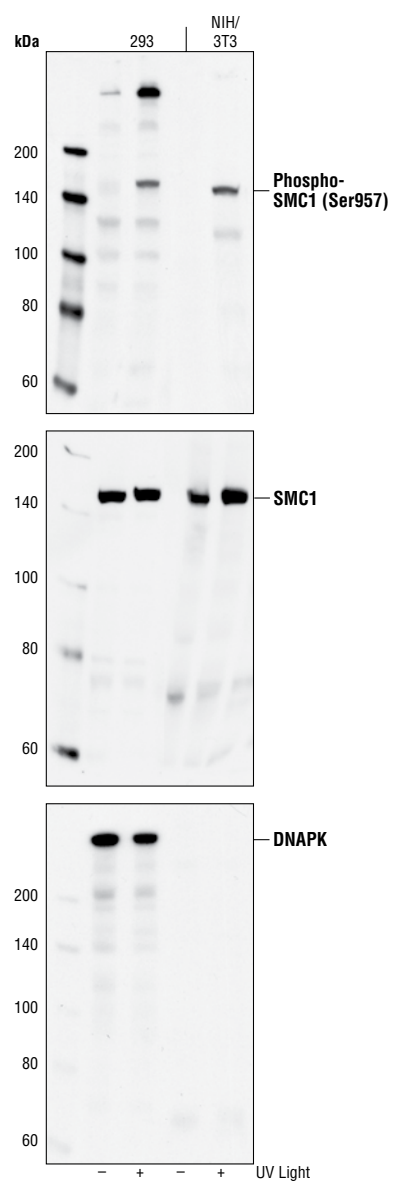
Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W	H, M, B	145 kDa	Mouse	IgG1

Background: Structural maintenance of chromosomes 1 (SMC1) protein is a chromosomal protein member of the cohesin complex that enables sister chromatid cohesion and plays a role in DNA repair (1,2). In response to ionizing radiation (IR), as part of the intra-S-phase DNA damage checkpoint, ATM/NBS1-dependent phosphorylation of SMC1 occurs at Ser957 and Ser966 (3). In cells subjected to other forms of DNA damage, including UV light and hydroxyurea treatment, SMC1 phosphorylation is ATM-independent (4). While phosphorylation of SMC1 is required for activation of the IR-induced intra-S-phase checkpoint, the precise mechanism is not well understood and may involve a conformational change that affects SMC1-SMC3 interaction (3).

Specificity/Sensitivity: Phospho-SMC1 (Ser957) (5D11G5) Mouse Monoclonal Antibody detects endogenous levels of SMC1 only when phosphorylated at serine 957. This antibody may also recognize phosphorylated human DNA-PKcs (450kDa).

Source/Purification: Monoclonal antibodies are produced by immunizing mice with a synthetic phosphopeptide corresponding to residues surrounding Ser957 of human SMC1.

- Background References:**
- (1) Michaelis, C. et al. (1997) *Cell* 91, 35–45.
 - (2) Sjogren, C. and Nasmyth, K. (2001) *Curr. Biol.* 11, 991–995.
 - (3) Yazdi, P.T. et al. (2002) *Genes Dev.* 16, 571–582.
 - (4) Kim, S.T. et al. (2002) *Genes Dev.* 16, 560–570.



Western blot analysis of extracts from 293 and NIH/3T3 cells, untreated or UV-treated, using Phospho-SMC1 (Ser957) (5D11G5) Mouse Monoclonal Antibody (upper), SMC1 Antibody #4802 (middle), and DNA-PK Antibody #4602 (lower).

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Entrez-Gene ID # 8243
Swiss-Prot Acc. # Q14683

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.**

Recommended Antibody Dilutions:
 Western blotting 1:1000

- Companion Products:**
- Phospho-SMC1 (Ser957) Antibody #4801
 - SMC1 Antibody #4802
 - Phospho-ATM (Ser1981) (10H11.E12) Mouse mAb #4526
 - Phospho-(Ser/Thr) ATM/ATR Substrate Antibody #2851
 - DNA-PK Antibody #4602
 - Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072
 - Anti-mouse IgG, HRP-linked Antibody #7076
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 μ l Tween-20 (100%).
9. **Phototope[®]-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
10. Prestained Protein Marker, Broad Range (Premixed Format) #7720
11. Biotinylated Protein Ladder Detection Pack #7727
12. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 μ l per well of 6-well plate or 500 μ l per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 μ l sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ l onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 μ l/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 μ l/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.