

HSP27 Antibody Sampler Kit



✓ 1 Kit
 (4 x 40 µl)

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

New 07/13

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-HSP27 (Ser82) (D1H2) XP® Rabbit mAb	9709	40 µl	27 kDa	Rabbit IgG
HSP27 (G31) Mouse mAb	2402	40 µl	27 kDa	Mouse IgG1
Phospho-HSP27 (Ser15) Antibody	2404	40 µl	27 kDa	Rabbit IgG
Phospho-HSP27 (Ser78) Antibody	2405	40 µl	27 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The HSP27 Antibody Kit provides an economical means to evaluate the activation status of the HSP27 protein. The kit contains enough primary antibody to perform four western blot experiments per primary antibody.

Background: Heat shock protein (HSP) 27 is one of the small HSPs that are constitutively expressed at different levels in various cell types and tissues. Like other small HSPs, HSP27 is regulated at both the transcriptional and posttranslational levels (1). In response to stress, the HSP27 expression increases several-fold to confer cellular resistance to the adverse environmental change. HSP27 is phosphorylated at Ser15, Ser78, and Ser82 by MAPKAPK-2 as a result of the activation of the p38 MAP kinase pathway (2,3). Phosphorylation of HSP27 causes a change in its tertiary structure, which shifts from large homotypic multimers to dimers and monomers (4). It has been shown that phosphorylation and increased concentration of HSP27 modulates actin polymerization and reorganization (5,6).

Specificity/Sensitivity: Each antibody in the HSP27 Antibody Sampler Kit recognizes endogenous levels of its specific target. Activation state antibodies detect their intended targets only when phosphorylated at the indicated site.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser82 of human HSP27 and to full-length human HSP27 protein. Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser15 or Ser78 of human HSP27 protein. Antibodies are purified by protein A and peptide affinity chromatography.

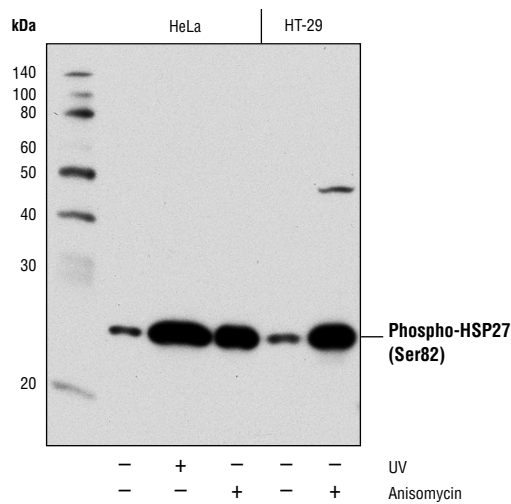
Background References:

- (1) Arrigo, A.P. and Landry, J. (1994) *Cold Spring Harbor Laboratory Press, NY*, 335-373.
- (2) Landry, J. et al. (1992) *J. Biol. Chem.* 267, 794-803.
- (3) Rouse, J. et al. (1994) *Cell* 78, 1027-1037.
- (4) Rogalla, T. et al. (1999) *J. Biol. Chem.* 274, 18947-18956.
- (5) Lavoie, J. et al. (1993) *J. Biol. Chem.* 268, 24210-24214.
- (6) Rousseau, S. et al. (1997) *Oncogene* 15, 2169-2177.

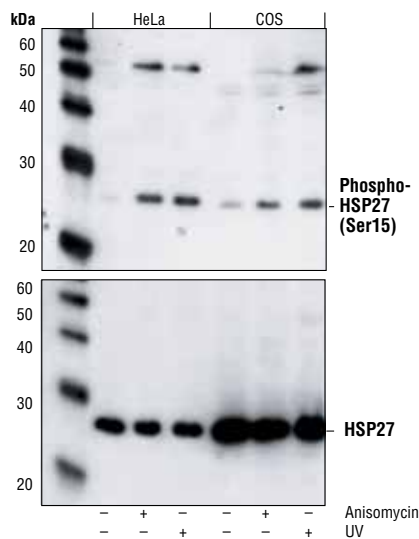
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
 Western blotting 1:1000

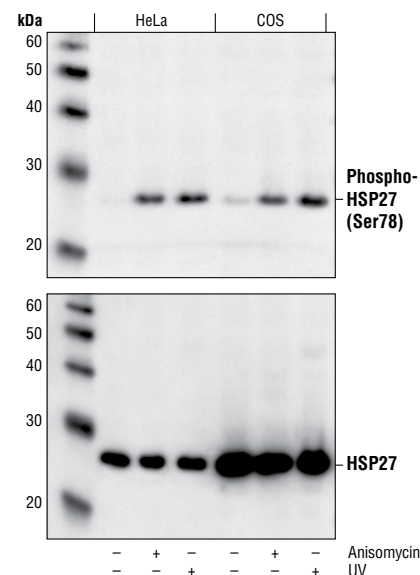
Please visit www.cellsignal.com for a complete listing of recommended companion products.



Western blot analysis of extracts from HeLa and HT-29 cells, untreated (-) or treated (+) with either UV (40 mJ/cm² with 30 min recovery) or Anisomycin #2222 (25 µg/mL, 30 min), using **Phospho-HSP27 (Ser82) (D1H2) XP® Rabbit mAb #9709**.



Western blot analysis of extracts from HeLa and COS cells, untreated (-) or treated with Anisomycin #2222 or UV (+), using **Phospho-HSP27 (Ser15) Antibody #2404** (upper) or **HSP27 (G31) Mouse mAb #2402** (lower).



Western blot analysis of extracts from HeLa and COS cells, untreated (-) or treated with Anisomycin #2222 or UV (+), using **Phospho-HSP27 (Ser78) Antibody #2405** (upper) or **HSP27 (G31) Mouse mAb #2402** (lower).

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

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

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

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

- 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.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- 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.

- 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.

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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- 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 µl Tween-20 (100%).
- 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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl 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.
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- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
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