

HSP27 Antibody Sampler Kit



✓ 1 Kit
(4 x 20 µl)

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rev 06/16

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	20 µl	27 kDa	Rabbit IgG
HSP27 (G31) Mouse mAb	2402	20 µl	27 kDa	Mouse IgG1
Phospho-HSP27 (Ser15) Antibody	2404	20 µl	27 kDa	Rabbit IgG
Phospho-HSP27 (Ser78) Antibody	2405	20 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 includes enough antibody to perform two western blot experiments with each 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.

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 validation data and a complete listing of recommended companion products.

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.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 1. 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 2. 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 3. 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
- 4. 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 5. 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH₂O, mix.
- 6. 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- 7. Nonfat Dry Milk:** (#9999)
- 8. Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- 9. Wash Buffer:** (#9997) 1X TBST
- 10. Bovine Serum Albumin (BSA):** (#9998)
- 11. Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- 12. Biotinylated Protein Ladder Detection Pack:** (#7727)
- 13. Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- 14. Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- 15. Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- 16. Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

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.

I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.
NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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