#9965 Store at –20°C

HSP/Chaperone Antibody Sampler Kit

☑ 1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
HSP60 (D6F1) XP® Rabbit mAb	12165	20 µl	60 kDa	Rabbit IgG
HSP70 Antibody	4872	20 µl	72, 73 kDa	Rabbit IgG
HSP40 (C64B4) Rabbit mAb	4871	20 µl	40 kDa	Rabbit IgG
HSP90 (C45G5) Rabbit mAb	4877	20 µl	90 kDa	Rabbit IgG
Calnexin (C5C9) Rabbit mAb	2679	20 µl	90 kDa	Rabbit IgG
PDI (C81H6) Rabbit mAb	3501	20 µl	57 kDa	Rabbit IgG
HSF1 Antibody	4356	20 µl	82 kDa	Rabbit IgG
BiP (C50B12) Rabbit mAb	3177	20 µl	78 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

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

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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.

Description: The HSP/Chaperone Sampler Kit provides an economical means to investigate protein folding within the cell. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: HSP70 and HSP90 are molecular chaperones expressed constitutively under normal conditions to maintain protein homeostasis and are induced upon environmental stress (1). HSP70 and HSP90 interact with unfolded proteins to prevent irreversible aggregation and catalyze the refolding of their substrates in an ATP-dependent manner (1). HSP40 family proteins bind unfolded proteins and prevent their aggregation, and deliver unfolded protiens to HSP70 (2). HSP60 has primarily been known as a mitochondrial protein that is important for folding key proteins after import into the mitochondria (3). HSP60 is also present in the cytosol of many cells and is induced by stress, inflammatory and immune responses, autoantibodies correlated with Alzheimer's, coronary artery diseases, MS, and diabetes (4-7). Secretory and transmembrane proteins are synthesized on polysomes and translocate into the endoplasmic reticulum (ER) where they are often modified by the formation of disulfide bonds, amino-linked glycosylation and folding. The ER contains a pool of molecular chaperones including calnexin, BiP and protein disulfide isomerase (PDI). Calenxin is a calcium-binding protein embedded in the ER membrane that retains newly synthesized glycoproteins inside the ER to ensure proper folding and quality control (8,9). When protein folding is disturbed inside the ER, Bip synthesis is increased. Subsequently, BiP binds to misfolded proteins to prevent them from forming aggregates and assists them to refold properly (10). PDI catalyzes the formation and isomerization of disulfide bonds required to reach a proteins native state (11). Heat shock gene transcription is regulated by a familly of heat shock factors (HSFs), transcriptional activators that bind to heat shock response elements (HSEs) located upstream of all heat shock genes (12). During attenuation from the heat shock response, HSF1 is repressed by direct binding of HSP70, HSP40/Hdj-1 and HSF binding protein 1 (HSBP1) (13).

Specificity/Sensitivity: HSP40 (C64B4) Rabbit mAb detects endogenous levels of total HSP40 protein. HSP60 (D6F1) XP® Rabbit mAb recognizes endogenous levels of total HSP60 protein. HSP70 Antibody detects endogenous levels of total HSP70 protein (HSP70-Hom, HSP70-1). HSP90 (C45G5) Rabbit mAb detects endogenous levels of total HSP90 protein. HSF1 Antibody detects endogenous levels of total HSF1 protein. Calnexin (C5C9) Rabbit mAb detects endogenous levels of total calnexin protein. PDI (C81H6) Rabbit mAb detects endogenous levels of total PDI protein. BiP (C50B12) Rabbit mAb detects endogenous levels of total BiP protein. Each of these antibodies recognizes only its specific target.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to human HSP70, and corresponding to residues at the carboxy-terminus of human HSF1 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Rabbit monclonal antibodies are produced by immunizing rabbits with a synthetic peptide corresponding to residues surrounding Trp68 of human HSP60 protein, residues surrounding Gly584 of human BiP, surrounding Asn300 of HSP90, corresponding to Glu223 of human HSP40/Hdj1, corresponding to the sequence of human calnexin, and corresponding to the sequence of human PDI.

Background References:

(1) Nollen, E.A. and Morimoto, R.I. (2002) *J. Cell Sci.* 115, 2809–2816.
(2) Fan, C.Y. et al. (2003) *Cell Stress Chaperones* 8, 309–316.
(3) Jindal, S. et al. (1989) *Mol. Cell. Biol.* 9, 2279–2283.
(4) Itoh, H. et al. (2002) *Eur. J. Biochem.* 269, 5931–5938.
(5) Gupta, S. and Knowlton, A.A. *J. Cell Mol. Med.* 9, 51–58.
(6) Deocaris, C.C. et al. (2006) *Cell Stress Chaperones* 11, 116–128.
(7) Lai, H.C. et al. (2007) *Am. J. Physiol. Endocrinol. Metab.* 292, E292–E297.
(8) Bergeron, J.J. et al. (1994) *Trends Biochem. Sci.* 19, 124–128.
(9) Williams, D.B. (2006) *J. Cell Sci.* 119, 615–623.
(10) Kohno, K. et al. (1993) *Mol. Cell. Biol.* 13, 877–890.
(11) Ellgaard, L. and Ruddock, L.W. (2005) *EMBO Rep.* 6, 28–32.
(12) Morimoto, R.I. (1998) *Genes Dev.* 12, 3788–3796.
(13) Satyal, S.H. et al. (1998) *Genes Dev.* 12, 1962–1974.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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 dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, 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 dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, 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 dH_2O, 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 dH_2O, 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.

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 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.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. 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.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

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

II. Primary Antibody Incubation

1. 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.
2. Wash three times for 5 min each with 15 ml of TBST.
3. 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.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. 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.
2. 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.