

HSP/Chaperone Antibody Sampler Kit

✓ 1 Kit
(8 x 40 microliters)

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

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
HSP60 (D307) Antibody	4870	40 µl	60 kDa	Rabbit IgG
HSP70 Antibody	4872	40 µl	72, 73 kDa	Rabbit IgG
HSP40 (C64B4) Rabbit mAb	4871	40 µl	40 kDa	Rabbit IgG
HSP90 (C45G5) Rabbit mAb	4877	40 µl	90 kDa	Rabbit IgG
Calnexin (C5C9) Rabbit mAb	2679	40 µl	90 kDa	Rabbit IgG
PDI (C81H6) Rabbit mAb	3501	40 µl	57 kDa	Rabbit IgG
HSF1 Antibody	4356	40 µl	82 kDa	Rabbit IgG
BiP (C50B12) Rabbit mAb	3177	40 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.

Description: The HSP/Chaperone Sampler Kit provides an economical means to investigate protein folding within the cell. The kit contains enough primary and secondary antibodies to perform four Western mini-blot experiments with each 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 proteins 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). Calnexin 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 family 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 (D307) Antibody detects 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 rabbits with a synthetic peptide (KLH-coupled) corresponding to Asp307 of human HSP60, 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 monoclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the sequence around Gly584 of human BiP, surrounding Asn300 of HSP90, corresponding to Glu223 of human HSP40/Hdj1, derived from the sequence of human calnexin, and corresponding to the sequence of human PDI.

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:

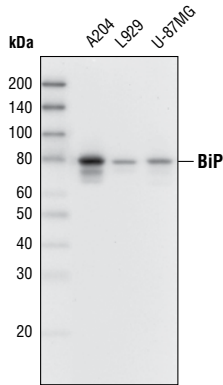
Western blotting 1:1000

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

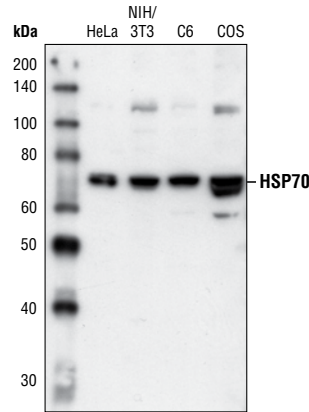
Background References:

- (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.

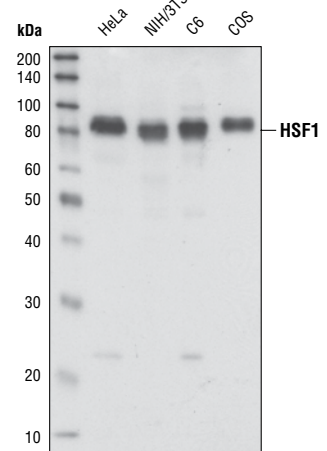
Name	Alternate name	Cellular Location	Co-Chaperones	Function
HSP40	DnaJ, HDJ1	Cytoplasm, Nucleus		HSP70 co-chaperone
HSP60	GroEL	Mitochondria	Chaperonin 10/GroES	Protein folding
HSP70	HSPA1A, DnaK	Cytoplasm, Nucleus	HSP40/DnaJ, Hip, Bag1, Hop	Protein folding
HSP90		Cytoplasm	Cdc37, Hop	Binding and stabilization of nascent polypeptide chains
HSF1	HSTF1	Cytoplasm, Nucleus		Activates heat shock protein genes following heat stress
Calnexin	CNX, IP90, p90	Endoplasmic reticulum		Mediates retention of misfolded proteins in the ER
PDI	PDIA1, P4HB	Endoplasmic reticulum		Catalyzes formation of disulfide bonds
BIP	GRP78, HSPA5	Endoplasmic reticulum	HEDJ	HSP70 family member, protein folding in ER



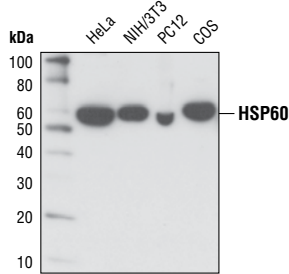
Western blot analysis of extracts from various cell types using **BIP (C50B12) Rabbit mAb #3177**.



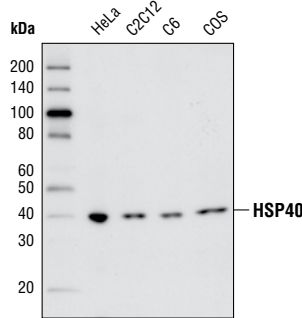
Western blot analysis of extracts from various cell types using **HSP70 Antibody #4872**.



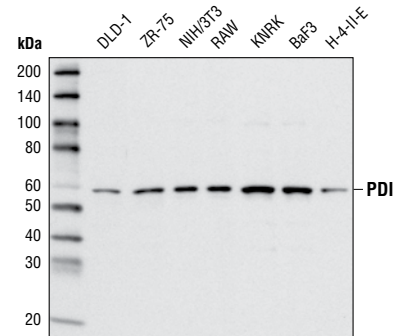
Western blot analysis of extracts from various cell types using **HSF1 Antibody #4356**.



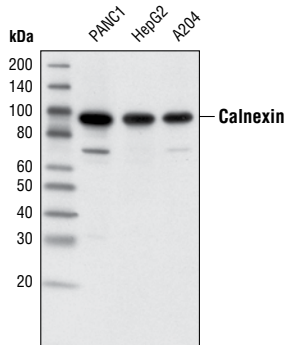
Western blot analysis of extracts from various cell types using **HSP60 (D307) Antibody #4870**.



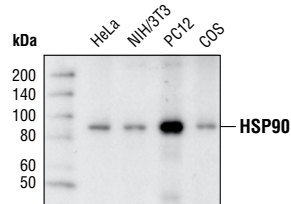
Western blot analysis of extracts from HeLa, C2C12, C6 and COS cells using **HSP40 (C64B4) Rabbit mAb #4871**.



Western blot analysis of extracts from various cell types using **PDI (C81H6) Rabbit mAb #3501**.



Western blot analysis of extracts from PANC1, HepG2 and A204 cells using **Calnexin (C5C9) Rabbit mAb #2679**.



Western blot analysis of extracts from HeLa, NIH/3T3, PC12 and COS cells using **HSP90 (C45G5) Rabbit mAb #4877**.

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.

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. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

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.