

**#4871** Store at -20°C

# HSP40 (C64B4) Rabbit mAb

100 µl  
 (10 Western mini-blot)



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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, IHC-P Endogenous	H, M, R, Mk	40 kDa	Rabbit**	IgG

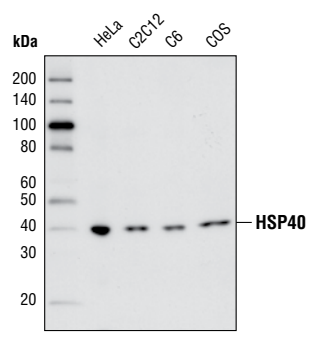
**Background:** HSP40 and HSP40-like proteins represent a large family of chaperone proteins that are homologous to *E. coli* DnaJ protein (1). These proteins are classified into three subtypes based on their structures. The common feature of the family is a conserved J domain, which is usually located at the amino terminus of proteins and responsible for their association with HSP70 (1,2). Human HSP40, also known as Hdj1, belongs to subtype II that contain a unique Gly/Phe-rich region (2). HSP40 family proteins bind unfolded proteins, prevent their aggregation, and then deliver them to HSP70 (2,3). Another major function of HSP40 is to stimulate ATPase activity of HSP70, which causes conformational change of the unfolded proteins (4,5). The HSP40-HSP70-unfolded protein complex further binds to co-chaperones Hip, Hop and HSP90 or components of the protein degradation machinery such as CHIP and BAG-1, which either leads to protein folding or degradation, respectively (6).

**Specificity/Sensitivity:** HSP40 (C64B4) Rabbit mAb detects endogenous levels of total HSP40 protein. This antibody does not cross-react with other HSPs.

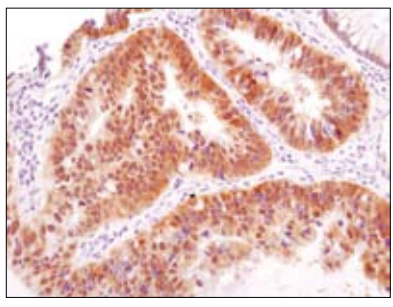
**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to Glu223 of human HSP40/Hdj1.

**Background References:**

- (1) Cheetham, M.E. and Caplan, A.J. (1998) *Cell Stress Chaperones* 3, 28–36.
- (2) Fan, C.Y. et al. (2003) *Cell Stress Chaperones* 8, 309–316.
- (3) Langer, T. et al. (1992) *Nature* 356, 683–689.
- (4) Liberek, K. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 2874–2878.
- (5) Cyr, D.M. et al. (1992) *J. Biol. Chem.* 267, 20927–20931.
- (6) Höhfeld, J. et al. (2001) *EMBO Rep.* 2, 885–890.



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



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using HSP40 (C64B4) Rabbit mAb.

**Entrez-Gene ID #** 3337  
**Swiss-Prot Acc. #** P25685

**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.**

**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**

Western blotting 1:1000  
 Immunohistochemistry (Paraffin) 1:25  
**IHC Protocol:** Unmasking buffer/wash buffer Citrate/TBST

**Companion Products:**

- HSP40 Antibody #4868
- HSP70 (6B3) Rat mAb #4873
- HSP70 (D69) Antibody #4876
- HSP70 Antibody #4872
- HSP90 (E289) Antibody #4875
- HSP90 Antibody #4874
- Hip Antibody #2723
- Hop Antibody #4464
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: 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.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
 F—Flow cytometry E—ELISA D—DELFIAP®  
 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 (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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.

## Immunohistochemistry Protocol (Paraffin)

**\*IMPORTANT:** See product data sheet for the appropriate wash buffer and antigen unmasking procedure. **IHC Protocol:** Unmasking buffer/wash buffer.

### A Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH<sub>2</sub>O)
4. Hematoxylin (optional)
5. **\*Wash Buffer:**
  - a. **PBST:** 1X PBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X PBS to 900 ml dH<sub>2</sub>O. Add 1ml Tween-20 and mix.  
**10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na<sub>2</sub>HPO<sub>4</sub>) and 2.4 g potassium phosphate, monobasic (KH<sub>2</sub>PO<sub>4</sub>) to 1 L dH<sub>2</sub>O. Adjust pH to 7.4.
  - b. **TBST:** 1X TBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X TBS to 900 ml dH<sub>2</sub>O. Add 1 ml Tween-20 and mix.  
**10X Tris Buffered Saline (TBS):** To prepare 1 L add 24.2 g Trizma® base (C<sub>4</sub>H<sub>11</sub>NO<sub>3</sub>) and 80 g sodium chloride (NaCl) to 1 L dH<sub>2</sub>O. Adjust pH to 7.6 with concentrated HCl.
6. **\*Antigen Unmasking Solution:**
  - a. **Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>•2H<sub>2</sub>O) to 1 L dH<sub>2</sub>O. Adjust pH to 6.0.
  - b. **EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>8</sub>Na<sub>2</sub>•2H<sub>2</sub>O) to 1 L dH<sub>2</sub>O. Adjust pH to 8.0.
  - c. **Alternative Unmasking: 10 mM Tris:** To prepare 1 L add 1.21 g Trizma® Base (C<sub>4</sub>H<sub>11</sub>NO<sub>3</sub>) to 1 L dH<sub>2</sub>O. Adjust pH to 10.0.
  - d. **Pepsin:** 1 mg/ml in Tris-HCl pH 2.0.
7. **3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H<sub>2</sub>O<sub>2</sub> to 90 ml dH<sub>2</sub>O.
8. **Blocking Solution:** 5% horse serum or goat serum diluted in recommended wash buffer.
9. Biotinylated secondary antibody.
10. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
11. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

### B Deparaffinization/Rehydration

**NOTE:** Do not allow slides to dry at any time during this procedure.

**NOTE:** Consult product data sheet for recommended wash buffer.

1. **Deparaffinize/hydrate sections:**
  - a. Incubate sections in three washes of xylene for 5 minutes each.
  - b. Incubate sections in two washes of 100% ethanol for 10 minutes each.
  - c. Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH<sub>2</sub>O for 5 minutes each.

### C \*Antigen Unmasking

**NOTE:** Consult product data sheet for specific recommendation for the unmasking solution.

1. **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
2. **For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.
3. **Alternate:** Bring slides to a boil in 10 mM Tris pH 10.0 followed by 10 minutes at a sub-boiling temperature. Cool slides on bench top for 30 minutes.
4. **For Pepsin:** Digest for 10 minutes at 37°C.

### D Staining

1. Wash sections in dH<sub>2</sub>O three times for 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections in dH<sub>2</sub>O twice for 5 minutes each.

**NOTE:** Consult product data sheet for recommended wash buffer.

4. Wash section in wash buffer for 5 minutes.
5. Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100-400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
7. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
8. Add 100-400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
9. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
10. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
11. Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
12. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
13. Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
14. As soon as the sections develop, immerse slides in dH<sub>2</sub>O.
15. If desired, counterstain sections in hematoxylin per manufacturer's instructions.
16. Wash sections in dH<sub>2</sub>O two times for 5 minutes each.
17. Dehydrate sections:
  - a. Incubate sections in 95% ethanol two times for 10 seconds each.
  - b. Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
  - c. Repeat in xylene, incubating sections two times for 10 seconds each.
18. Mount coverslips.