

Store at
-20°C

#89947

ER Stress-induced Autophagy Antibody Sampler Kit

1 Kit (7 x 20 µl)



Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
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New 02/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Atg12 (D88H11) Rabbit mAb	4180	20 µl	16, 55 kDa	Rabbit IgG
Beclin-1 (D40C5) Rabbit mAb	3495	20 µl	60 kDa	Rabbit IgG
BiP (C50B12) Rabbit mAb	3177	20 µl	78 kDa	Rabbit IgG
eIF2α (D7D3) XP® Rabbit mAb	5324	20 µl	38 kDa	Rabbit IgG
JNK1 (2C6) Mouse mAb	3708	20 µl	46, 54 kDa	Mouse IgG1
Phospho-eIF2α (Ser51) (D9G8) XP® Rabbit mAb	3398	20 µl	38 kDa	Rabbit IgG
Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb	4668	20 µl	46, 54 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 ER Stress-induced Antibody Sampler Kit contains reagents to investigate ER stress-induced signaling within the cell. The kit contains enough primary antibodies to perform two western blot experiments per primary antibody.

Background: The endoplasmic reticulum (ER) is an organelle with essential biosynthetic and signaling functions in eukaryotic cells (1). Post synthesis of secretory and transmembrane proteins on polysomes, proteins are translocated into the ER where they are often modified by disulfide bond formation, amino-linked glycosylation, and folding. Different physiological and pathological conditions can disturb proper protein folding in the ER causing ER stress (1). ER stress activates an intracellular signaling transduction pathway called unfolded protein response (UPR) and autophagy to avoid cell death (2). The main role of UPR is to improve the protein load on the ER by shutting down protein translation and gene transcription to enhance ER's folding capacity (2). On the other hand, autophagy is a catabolic process for the autophagosome-lysosomal degradation of bulk cytoplasmic contents (3,4). One of the chaperones aiding in proper protein folding is Binding immunoglobulin Protein (BiP) (5,6). BiP works by binding to misfolded proteins to prevent them from forming aggregates and assists in proper refolding (7). The molecular machinery of autophagy was largely discovered in yeast and referred to as autophagy-related (Atg) genes. Formation of the autophagosome involves a ubiquitin-like conjugation system in which Atg12 is covalently bound to Atg5 and targeted to autophagosome vesicles (8-10). One of the proteins critical to autophagy process is Beclin-1, the mammalian orthologue of the yeast autophagy protein Apg6/Vps30 (11). Beclin-1 can complement defects in yeast autophagy caused by loss of Apg6 and can also stimulate autophagy when overexpressed in mammalian cells (12). Mammalian Beclin-1 was originally isolated in a yeast two-hybrid screen for Bcl-2 interacting proteins and has been

shown to interact with Bcl-2 and Bcl-xL, but not with Bax or Bak (13). Phosphorylation of the eukaryotic initiation factor 2 (eIF2) α subunit is a well-documented mechanism to downregulate protein synthesis under a variety of stress conditions. eIF2 binds GTP and Met-tRNAi and transfers Met-tRNA to the 40S subunit to form the 43S preinitiation complex (14,15). Kinases that are activated by viral infection (PKR) can phosphorylate the α subunit of eIF2 (16,17). Induction of PKR by IFN-γ and TNF-α induces potent phosphorylation of eIF2α at Ser51 (18,19). There are three SAPK/JNK genes each of which undergoes alternative splicing, resulting in numerous isoforms (20). The IRE1, a transmembrane serine/threonine kinase (21,22), through its kinase activity activates SAPK/JNK in the early stage of ER stress in order to induce autophagosome formation (23).

Specificity/Sensitivity: Each antibody in the ER Stress-induced Antibody Sampler Kit detects endogenous levels of its target protein. Phospho-eIF2α (Ser51) (D9G8) XP® Rabbit mAb detects endogenous eIF2α only when phosphorylated at Ser51. The antibody does not recognize eIF2α phosphorylated at other sites. Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb detects endogenous levels of p46 and p54 SAPK/JNK only when phosphorylated at Thr183 and Tyr185. This antibody may cross-react with phosphorylated p44/42 or p38 MAP kinases. JNK1 (2C6) Mouse mAb detects endogenous levels of total JNK1 protein. This antibody may cross react with recombinant levels JNK2 protein. The antibody does not cross react with JNK3 protein.

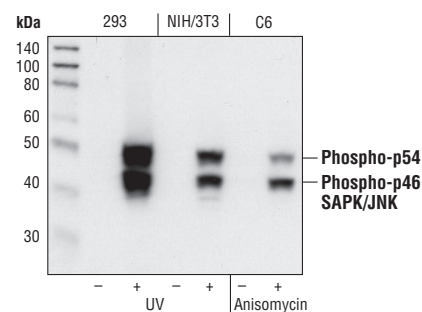
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:

Western blotting 1:1000

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly584 of human BiP, residues surrounding Ser36 of human Atg12 protein, residues surrounding Thr72 of human Beclin-1, residues surrounding Ser51 of human eIF2α, residues surrounding Thr183/Tyr185 of human SAPK/JNK, residues of a purified recombinant human eIF2α, and residues corresponding to the amino terminus of human JNK1.



Western blot analysis of extracts from 293 cells, untreated or UV-treated, NIH/3T3 cells, untreated or UV-treated and C6 cells, untreated or anisomycin-treated, using **Phospho-SAPK/JNK (Thr183/Tyr185) (81E11) Rabbit mAb #4668**.

U.S. Patent No. 5,675,063

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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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 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.
- 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.
- 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.
- 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.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- 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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