Revision 1

Store at -20C

#89947

ER Stress-induced Autophagy Antibody Sampler Kit

1 Kit (7 x 20 microliters)

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
BiP (C50B12) Rabbit mAb	3177	20 µl	78 kDa	Rabbit IgG
eIF2α (D7D3) XP® Rabbit mAb	5324	20 µl	38 kDa	Rabbit IgG
Phospho-eIF2α (Ser51) (D9G8) XP® Rabbit mAb	3398	20 µl	38 kDa	Rabbit IgG
Atg12 (D88H11) Rabbit mAb	4180	20 µl	16, 55 kDa	Rabbit IgG
Beclin-1 (D40C5) Rabbit mAb	3495	20 µl	60 kDa	Rabbit IgG
JNK1 (2C6) Mouse mAb	3708	20 µl	46, 54 kDa	Mouse IgG1
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

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

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 four western blot experiments per primary antibody.

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.

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 autophagosomic-lysosomal degradation of bulk cytoplasmc 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).

Background References

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