

ORDERING INFORMATION

Catalog Number: MAB3024

Clone: 333116

Lot Number: XAV02

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

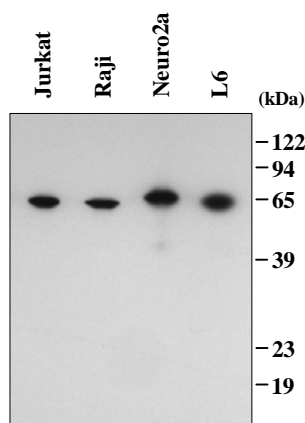
Storage: -20° C

Specificity: human/mouse/rat Keap1

Immunogen: *E. coli*-derived rhKeap1

Ig class: mouse IgG_{2b}

Recommended Application:
Western blot



Detection of Keap1 with MAB3024.

Lysates from human Jurkat and Raji, mouse Neuro2A and rat L6 cells were resolved by SDS-PAGE, transferred to Immobilon-P membrane and immunoblotted with 1.0 µg/mL mouse anti-Keap1 as described in *Protocols for Immunoblotting*. A 30 second exposure to film is shown.

Background

Kelch-like ECH-associated protein 1 (Keap1) is a 624 amino acid, 69 kDa protein which interacts with the transcription factor NF-E2-related factor 2 (Nrf2). Keap1 represses Nrf2 function by sequestering Nrf2 in the cytoplasm. Keap1 contains an N-terminal BTB domain and six C-terminal KELCH domains (aa 327 - 611) that interact with Nrf2. Dissociation of the two proteins in response to redox-sensitive cell stress is followed by the translocation of Nrf2 to the nucleus and transcription of detoxifying/oxidative stress enzyme genes.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived recombinant human Keap 1 (rhKeap1; aa 90 - 250; Accession # NP_036421). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody is known to react with endogenous human, mouse and rat Keap1 on Western blots.

Application

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Optimal dilutions should be determined by each laboratory for each application.

Protocols for Immunoblotting

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	2% nonfat dry milk	1% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween [®] 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1.0 µg/mL anti-human/mouse/rat Keap1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG-HRP (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Tween is a registered trademark of ICI Americas.