



Product Specification Sheet

Phospho Microtubule-associated proteins 1A/1B light chain 3B (LC3B) Antibody

□ Cat # LC3B21-A

Rabbit Anti-Mouse Phospho (Ser12) LC3B antibody

SIZE: 100 ug

Microtubule-associated proteins 1A/1B light chain 3B or LC3 is a central protein in the autophagy pathway where it functions in autophagy substrate selection and autophagosome biogenesis. LC3 is the most widely used marker of autophagosomes. LC3 is synthesized as Pro-LC3 which is processed by Atg4 into LC3-I. During autophagosome formation, LC3-I links to phosphatidylethanolamine and is incorporated into autophagosome membranes. This process converts LC3-I into an active, autophagosome membrane-bound form, LC3-II. In western blotting, LC3 is detected as two bands; cytosolic LC3-I and membrane bound LC3-II.

Source of Antigen or Antibodies

Uniprot: Q9CQV6

Host: Rabbit

Clonality: Polyclonal

Purification: Ammonium sulfate followed by peptide affinity purification

Immunogen: 16 amino acid phosphopeptide derived from Mouse LC3B surrounding Serine 12 conjugated to KLH

Species Reactivity: Mouse

Cross reactivity: Peptide region used as an immunogen shows 100% homology with Rat. 93% with Human, NHP, Dog, Bovine, and Horse

Tissue specificity: Most abundant in the heart, brain, skeletal muscle, and testis. Little expression is observed in the liver.

Subcellular Location: Cytoskeleton, endomembrane and autophagosome membrane

Alternative name: Autophagy-related protein LC3B, autophagy-related ubiquitin-like modifier LC3B, MAP1 light chain 3-like protein 2, MAP1A/MAP1B light chain 3 B.

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (**ADI cat#20320**)

Negative Control: Non-immune Rabbit IgG (**ADI cat# 20009-1**).

Form & Storage of Antibodies

Affinity pure IgG

□ Solution

Concentration: 0.5 mg/ml Volume: 200 ul
Supplied in PBS pH 7.4, 0.1% BSA,

Storage:

Long-term: at -20°C. Do not aliquot. Do not expose to multiple freeze/thaw cycles or store working, diluted solutions.

Recommended Usage

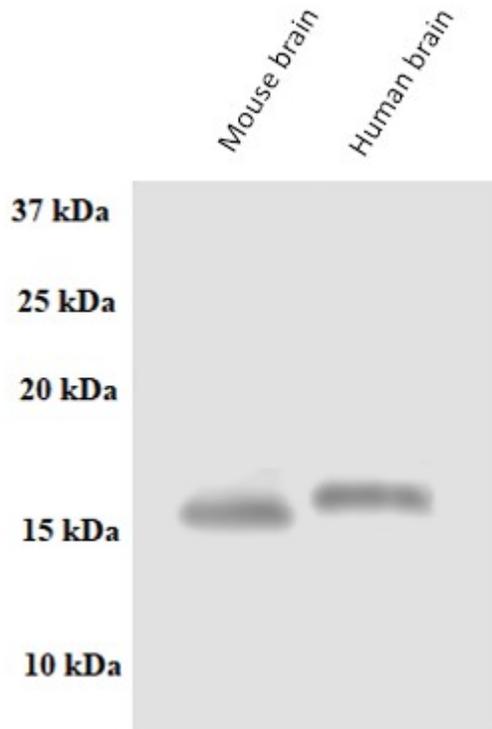
Western Blotting: 0.5-2 ug/ml
Theoretical band size: 14.6 kDa
Observed Band size: ~14 and 16 kDa.

*Above concentrations are a suggestion, user's must optimize their assay's based on their own conditions. The antibody may work in other applications. These methods have not been tested by ADI.

**This product is for In vitro research use only.*

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25 ug of Mouse brain and human brain lysates were heated for 5 minutes at 95°C then separated on an 'Any Kd' SDS-PAGE gel (Biorad) The gel was run for ~1 hr 30 minutes at 100V and transferred to a 0.2 um nitrocellulose membrane using the low MW settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with 1% Fish plasma (Aquablock, EastCoastBio). **LC3B21-A** was diluted with TBST+0.1% BSA to 1 ug/ml and incubated overnight at 4°C. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was then diluted 1:10,000 (50 ng/ml) in TBST+0.1% BSA and incubated for 1 hour at room temperature. The blot was washed with TBS-T 3 times for 5 minutes each. The blot was incubated with ADI Fento ECL substrate (**ADI cat#80210**) for 5 minutes and imaged on a LI-COR C-DIGIT at high sensitivity settings. Specific band is observed ~16 kDa.