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NB110-60928 Protocol

Serum protocol for ATG16L1 Antibody (NB110-60928)

[[URL:https://www.novusbio.com/products/atg16l1-antibody_nb110-60928]][[Caption:ATG16L1 Antibody]]
Protocol: Western Blot Protocol for Atg16L1 Antibody (NB110-60928)

Materials
1X PBS
Sample buffer, 2X Laemmli buffer: 4% SDS, 5% 2-mercaptoethanol (BME), 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl, pH 6.8
1X Running Buffer: 25 mM Tris-base, 192 mM glycine, 0.1% SDS. Adjust to pH 8.3
1X Transfer buffer (wet): 25 mM Tris-base, 192 mM glycine, 20% methanol Adjust to pH 8.3
TBS
TBST, TBS and 0.1% Tween
Blocking solution: TBST, 5% non-fat dry milk
rabbit anti-Atg16L1 primary antibody (NB110-60928) in blocking buffer (~2 ug/mL)

Methods

1. Grow cells (e.g. HeLa or Neuro2A) in vitro to semi-confluency (70-75%).
2. Rinse cells with ice-cold 1X PBS and lyse cells with sample buffer.
3. Sonicate and incubate cells for 5 minutes at 95oC.

Tip: Cells are lysed directly in sample buffer.

4. Load 10-40 ug/lane of sample on a 12% polyacrylamide gel (SDS-PAGE).
5. Transfer proteins to a PVDF membrane for 60 minutes at 100V.
Tip: For more information on Western Blotting, see our Western Blot handbook: https://images.novusbio.com/design/BR_westernblotguide_042816b.pdf
6. After transfer, rinse the membrane with dH2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.
7. Rinse the membrane in dH2O to remove excess stain and mark the loaded lanes and molecular weight markers using a pencil.
8. Block the membrane using blocking buffer solution (5% BSA in TBST) for 16 hours at 4oC.
9. Rinse the membrane with TBST for 5 minutes.

10. Dilute the rabbit anti-Atg16L1 primary antibody (NB110-60928) in blocking buffer (~2 ug/mL) and incubate the membrane for 1.5 hours at room temperature.
11. Rinse the membrane with dH2O.
12. Rinse the membrane with TBST, 3 times for 10 minutes each.
13. Incubate the membrane with diluted secondary antibody, according with product's specification, (e.g. anti-rabbit-IgG HRP-conjugated) in blocking buffer for 1 hour at room temperature.

Note: Tween-20 may be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.
14. Rinse the membrane with TBST, 3 times for 10 minutes each.
15. Apply the detection reagent of choice (e.g. BioFX Super Plus ECL) in accordance with the manufacturer's instructions.