Protocol: Western Blot Protocol for Atg5 Antibody (NB110-53818)

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-Atg5 primary antibody (NB110-53818) in blocking buffer (1:500)

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 95^{o}C.

   Tip: Cells are lysed directly in sample buffer.

4. Load 10-40 μg/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 dH_2O and stain with Ponceau S for 1-2 minutes to confirm efficiency of protein transfer.

7. Rinse the membrane in dH_2O 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 1 hour at room temperature.

9. Rinse the membrane with TBST for 5 minutes.

10. Dilute anti-Atg5 primary antibody (NB110-53818) in blocking buffer (1:500) and incubate the membrane for 1 hour at room temperature.

11. Rinse the membrane with dH_2O.

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.

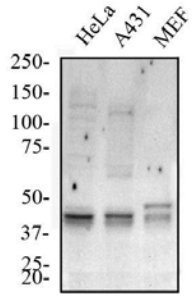
    Note: Tween-20 may be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

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.

16. Image the blot.

    Note: in some cell lines Atg5 and Atg12 predominantly exist in their conjugated form.



Western Blot: ATG5 Antibody [NB110-53818] - Total protein from Human HeLa and A431 and Mouse MEF cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 μg/mL anti-ATG5 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.