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NBP2-49888 Protocol

Western Blot protocol for Tat-Beclin 1 Autophagy Inducing Peptide (NBP2-49888)

WB Protocol for Tat-Beclin 1 L11 or Tat-Beclin 1 D11 Induced Autophagy in HeLa Cells

Important Notes
1. Peptides Tat-Beclin 1 L11 [NBP2-49886] and Tat-Beclin 1 D11 [NBP2-49888] are useful for induction of autophagy.
2. Tat-Beclin 1 L11S [NBP2-49887], an inactive/scrambled control peptide derived from Tat-Beclin 1 L11, is useful as a negative control when analyzing NBP2-49886 and/or NBP2-49888 for autophagy induction experiments.
3. Molecular weights for L11, L11S and D11 are 3.08 kDa each.
4. Tat-Beclin 1 D11 should NOT be reconstituted at a concentration greater than 5 mM.
5. An increased levels of LC3B-II band or a decreased levels of p62 indicate autophagy induction.

Cell Culture and Treatments
1. Plate HeLa cells overnight in 12 well plates and check for confluency. Cells should be 60-80 % confluent before treatments.
2. Wash cells 3 times with 1X PBS.
3. Re-suspend 1 mM of each peptide in OptiMEM (Life Technologies: 11058021) acidified with 0.15 % 6 N HCl.

Optimization of peptide concentration and incubation time
i. To determine the most effective concentration for your cell line perform a 1:2 serial dilution with the 1 mM peptides starting with 20 uM and diluting to 0 uM final concentration in each well.
ii. Duration of induction would be concentration and cell line dependent. HeLa cells may be incubated with the peptides up to 20 uM /up to 2 hrs.

Cell Lysate Preparation
4. Remove the medium from one well at each time point and add cold lysis buffer immediately. Cell lysis may be performed using 150 uL of M-PER (TM) Mammalian Protein Extraction Reagent (Thermo 78501) with 1:100 Halt (TM) Protease and Phosphatase Inhibitor Single-Use Cocktail (Thermo 78442) per well.
5. Incubate the plates at room temperature for 10 min with gentle agitation.
6. Scrape the cells from the plate and spin down at 13500 rpm for 10 min at 4C. Save the supernatant (lysate) and discard the pellet (cell debris).

Western Blot
7. Add 30 uL of 6X Lamelli Reducing SDS loading buffer to 150 uL of the lysate. Boil the solution for 5 min at 95C and then cool to room temperature before loading.
8. Use a 5-20% gradient gel and load the wells with 10 uL of reduced sample. Run the gel at 130V for 1 hour.
9. Transfer the proteins from gel to a nitrocellulose membrane at 100V for 1 hour.
10. Block the membranes for 1-2 hours at room temperature in Pierce (TM) Protein-Free (PBS) Blocking Buffer (Thermo 37584).
11. Incubate the membranes for overnight in blocking buffer with the respective antibodies: rabbit anti-LC3B (Novus NB100-2220) at 2 ug/mL, mouse anti-SQSTM1/p62 (Novus MAB8028) at 2 ug/mL, and sheep anti-actin (Novus AF4000) at 1 ug/mL.
12. Next day, rinse the membranes with DI water and wash with 1X TBST for 1 hour at room temperature. Probe the membranes with a secondary antibodies for 1 hour at room temperature; goat anti-rabbit IgG HRP (Novus HAF008) at 1:1000, donkey anti-mouse IgG HRP (Novus HAF018) at 1:1000, and donkey anti-sheep IgG HRP (Novus HAF016) at 1:1000.
13. Wash the membranes for 2 hours with 1X TBST and then develop using a 1:1 solution of WesternGlo A and B for 1 min with a 1 min exposure time on a Kodak Chemiluminescent imager.

Useful Resources:

1. Troubleshooting for Autophagy and LC3:-

2. Autophagy Research Sub-topics:-
3. Support by Application, Protocols:-