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NBP2-49888 Protocol

Immunocytochemistry/Immunofluorescence protocol for Tat-Beclin 1 Autophagy Inducing Peptide (NBP2-49888)

ICC/IF protocol to induce autophagy in HeLa cells using Tat-Beclin 1 L11 or Tat-Beclin 1 D11 peptides.

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 L11S [NBP2-49887], an inactive/scrambled control peptide derived from Tat-Beclin 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 D11 should NOT be reconstituted at a concentration greater than 5 mM.
5. Antibodies against LC3B or p62/SQSTM1 may be used for detecting the induction of autophagy. An increased number of LC3 stained vacuoles or decreased levels of p62 indicate autophagy induction.

Day 1 - Culturing HeLa Cells
1. Plate cells at a density of 1-1.5 x 10^5 cells per mL and 100 uL per well in DMEM with 10% FBS and 1X Pen/Strep into a black-welled Perkin Elmer Cell Carrier 96-well plate (6005550).
2. Incubate the cultured plate overnight at 37C with 5 % CO2.

Day 2 - Treatments
1. Check cells for confluency. Cells should be 60-80% confluent before treatments.
2. Wash cells 3 times with 1X PBS.
3. Resuspend 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 of interest, perform a 1:2 serial dilution with the 1 mM peptides. Start with at least 20 uM and dilute to 0 uM final testing concentration in each well.
ii. Duration of induction can be determined by collecting images at every 15 min up to 2 hrs. Fix the cells from one well at each time point according to the instructions starting from Step 6 below.
4. Add 50 uL to each well (in triplicate)
5. Incubate for 1.5 hrs at 37C with 5 % CO2.
6. Remove remaining liquid from wells and fix cells with 4% paraformaldehyde for 20 minutes at room temperature.
7. Wash cells 3 times with 1X PBS.
8. Block cells with blocking buffer (1X PBS, 0.1 % Triton-X, 5 % Normal Donkey Serum) for 1 hr at room temperature (alternately, cells can be blocked overnight at 4 C).

Day 2/3 - Primary Antibody Staining
1. Dilute the primary antibody in blocking buffer to the specifications listed in the antibody datasheet.
2. Incubate the primary antibodies overnight at 4 C or 2 hrs at room temperature.

Day 2/3 - Secondary Antibody Staining
1. Wash cells 3 times with 1X PBS.
2. Dilute the secondary antibodies to specifications in the blocking buffer.
3. Incubate for 1 hr at room temperature in dark.
4. Wash cells 3 times with 1X PBS.
5. Stain with DAPI, cytosolic stain [NeuroTrace (R)], and Northern Lights Guard (R&D Systems, Inc. NL996).
a. NeuroTrace (R)- 1:200 dilution in 1X PBS.
b. DAPI- 1:10000 dilution into NeuroTrace (R)/1X PBS solution.
c. Northern Lights Guard - add 1:1 to NeuroTrace (R)/DAPI/PBS solution
6. Leave solution in wells and seal with a foil plate sealer.
7. Image plate. Store plate at 4 C in dark.

Useful Resources:

1. Troubleshooting for Autophagy and LC3:
2. Autophagy Research Sub-topics:
3. Support by Application, Protocols: