Product Datasheet

Tat-Beclin 1 D11 Autophagy Inducing Peptide - Retroinverso form

NBP2-49888

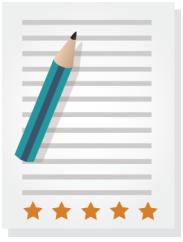
Unit Size: 1 mg

Store at -20C in powder form. Store at -80C once reconstituted.

Reviews: 1 Publications: 21

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NBP2-49888

Updated 3/7/2024 v.20.1



NBP2-49888

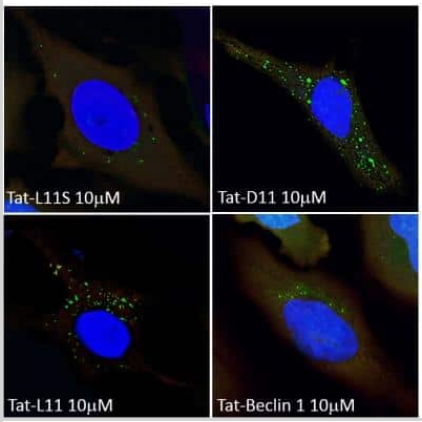
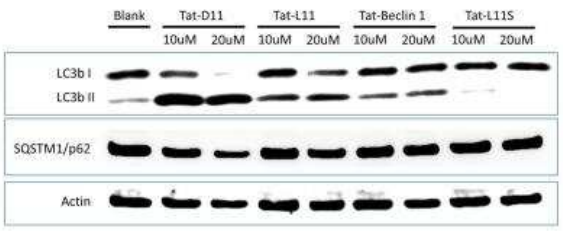
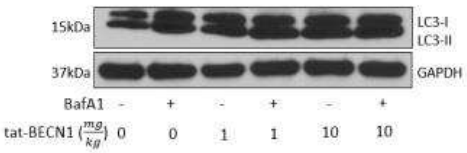
Tat-Beclin 1 D11 Autophagy Inducing Peptide - Retroinverso form

Product Information	
Unit Size	1 mg
Concentration	Lyoph
Storage	Store at -20C in powder form. Store at -80C once reconstituted.
Reconstitution Instructions	Reconstitute with DMSO or water to desired concetration. Note:- D11 should NOT be reconstituted at a concentration greater than 5 mM.
Buffer	This product is supplied lyophilized. Purity is >= to 97% (HPLC)
Target Molecular Weight	3.08 kDa

Product Description	
Description	Tat-D11 [NBP2-49888]: peptides comprising 11 amino acids derived from Beclin 1 linked to the HIV Tat protein with a diglycine linker. These peptides are in the retero-inverso D-configuration. The amino acid sequence of Tat-Beclin 1 D11 is RRRQRRKKRGYGGDHWIHFTANWV (Bio-Techne's exclusive patent license: US Patent 8,802,633).
Species	Non-species specific
Notes	Note: Tat-L11 and Tat-D11, are exclusively available from Novus Biologicals (Bio-Techne's exclusive patent license: US Patent 8,802,633).

Product Application Details	
Applications	Functional, In vitro assay, In vivo assay
Recommended Dilutions	Functional, In vitro assay, In vivo assay

Images

Immunocytochemistry/Immunofluorescence: Tat-Beclin 1 D11 Autophagy Inducing Peptide [NBP2-49888] - HeLa GFP-LC3B cells were treated with Tat-D11, Tat-L11, Tat-Beclin 1 or Tat-L11S for 1.5 hours. Thereafter, the cells were stained using NeuroTrace Red or DAPI and analyzed employing fluorescent microscopy. Note the higher number of autophagosomes/GFP-LC3B+ puncta in the images of Tat-D11 and Tat-L11 treated cells when compared to Tat-Beclin 1 and Tat-L11S treated cells.	
In vitro assay: Tat-Beclin 1 D11 Autophagy Inducing Peptide - Retroinverso form [NBP2-49888] - Analysis of lysates from HeLa cells that were left untreated (blank) or were treated with 10-20 uM each of Tat-D11, Tat-L11, Tat-Beclin 1 or Tat-L11S. The lysates were analyzed for the expression of LC3-1/LC3-II and SQSTM1/p62 using 2 ug/mL each of anti-LC3B (NB100-2220) and anti-SQSTM1/p62 (MAB8028) respectively. Anti-Actin (AF4000) was used as a loading control. TatD11 exhibited superior induction of LC3-II and down-regulation of p62 protein when compared to other treatment and control groups.	
In vivo assay: Tat-Beclin 1 D11 Autophagy Inducing Peptide - Retroinverso form [NBP2-49888] - In vivo dose study in 10wk old C57BL/6J mice. Either 1mg/kg or 10mg/kg IP once daily was administered for 2 days, mice were sacrificed, kidneys prepared for Western blot analysis. Lysosomal inhibitor bafilomycin A1 was used to provide a measurement of autophagic flux. *vehicle is scrambled tat-beclin (NBP2-49887-5mg). Image from verified customer review.	

Publications

Khang Nguyen, Jialing Tang, Sungji Cho, Fan Ying, Hye Kyoung Sung, James Wonsuk Jahng, Kostas Pantopoulos, Gary Sweeney Salubrinal promotes phospho-eIF2α-dependent activation of UPR leading to autophagy-mediated attenuation of iron-induced insulin resistance Molecular Metabolism 2024-03-26 [PMID: 38527647]

Sharif T, Martell E, Dai C et al. HDAC6 differentially regulates autophagy in stem-like versus differentiated cancer cells. Autophagy. 2018-11-16 [PMID: 30444165]

Ramesh B. Kasetti, Prabhavathi Maddineni, Charles Kiehlbauch, Shruti Patil, Charles C. Searby, Beth Levine, Val C. Sheffield, Gulab S. Zode Autophagy stimulation reduces ocular hypertension in a murine glaucoma model via autophagic degradation of mutant myocilin JCI Insight 2021-03-08 [PMID: 33539326]

Atsushi F, Tomoko S, Mihoko Y et al. Chloroquine is a safe autophagy inhibitor for sustaining the expression of antioxidant enzymes in trophoblasts Journal of Reproductive Immunology 2022-11-01 [PMID: 36470134]

Zheng N, Fang J, Xue G et al. Induction of tumor cell autosis by myxoma virus-infected CAR-T and TCR-T cells to overcome primary and acquired resistance Cancer cell 2022-08-15 [PMID: 36027915]

Soria L, Perocheau D, De Sabbata G et al. Beclin-1-mediated activation of autophagy improves proximal and distal urea cycle disorders EMBO Mol Med 2020-12-28 [PMID: 33369168]

Luis LB, Ana GT, Carlos GE et al. Salmonella Promotes Its Own Survival in B Cells by Inhibiting Autophagy Cells 2022-06-29 [PMID: 35805144] (In vitro)

Forte M, Bianchi F, Cotugno M Et al. An interplay between UCP2 and ROS protects cells from high-salt-induced injury through autophagy stimulation Cell death & disease 2021-10-08 [PMID: 34625529] (In vitro)

Pavel M, Park SJ, Frake RA et al. alpha-Catenin levels determine direction of YAP/TAZ response to autophagy perturbation Nature communications 2021-03-17 [PMID: 33731717]

Yang J, Kitami M, Pan H, et al. Augmented BMP signaling commits cranial neural crest cells to a chondrogenic fate by suppressing autophagic beta-catenin degradation Science signaling 2021-01-12 [PMID: 33436499]

Mathew B, Chennakesavalu M, Sharma M et al. Autophagy and post-ischemic conditioning in retinal ischemia Autophagy 2020-05-26 [PMID: 32452260] (In vitro, Rat)

Iaconis D, Crina C, Brillante S et al. The HOPS Complex Subunit VPS39 controls ciliogenesis through autophagy Hum. Mol. Genet. 2020-02-20 [PMID: 32077937] (Human)

More publications at http://www.novusbio.com/NBP2-49888

Procedures

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:-

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:

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Products Related to NBP2-49888

NBP2-49887	Tat-Beclin 1 L11S Peptide - Scrambled Control

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