Product Datasheet

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

Unit Size: 1 mg

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

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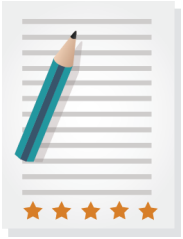
Updated 11/29/2020 v.20.1



NBP2-49887

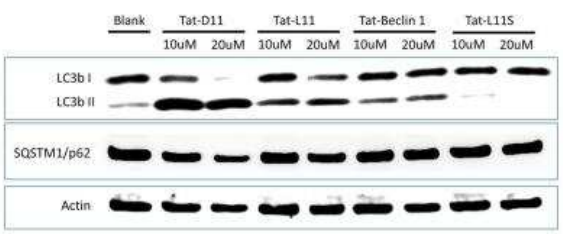
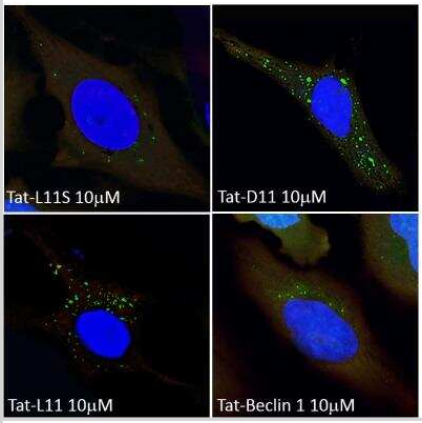
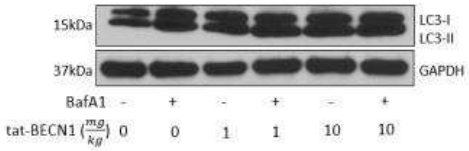
Tat-Beclin 1 L11S Peptide - Scrambled Control

Product Information	
Unit Size	1 mg
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C in powder form. Store at -80C once reconstituted.
Reconstitution Instructions	Reconstitute with DMSO or water to desired concetration.
Buffer	This product is supplied lyophilized. Purity is >= to 97% (HPLC)
Target Molecular Weight	3.08 kDa

Product Description	
Description	Tat-L11S [NBP2-49887]: inactive/scrambled control peptides derived from Tat-L11. These peptides are recommended as a negative control. The exact sequence of Tat-Beclin 1 L11S is YGRKKRRQRRRGGNWAWHDFVHIT (Bio-Techne's exclusive patent license: US Patent 8,802,633)
Species	Non-species specific

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

Images

Western Blot: Tat-Beclin 1 L11S Autophagy Inducing Peptide - Inactive Form [NBP2-49887] - WB 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 2ug/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.	
Immunocytochemistry/Immunofluorescence: Tat-Beclin 1 L11S Autophagy Inducing Peptide - Inactive Form [NBP2-49887] - 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 vivo assay: Tat-Beclin 1 L11S Peptide - Scrambled Control [NBP2-49887] - In vivo dose study in mouse kidneys as control for tat-beclin D11 autophagy inducing peptide. Well tolerated by mice at dose of 1mg/kg for 2 days IP. Soluble in PBS. First two lanes (controls) are treated with 1mg/kg tat-beclin scrambled peptide; remaining lanes are treated with tat-beclin D11 autophagy inducing peptide. Image from verified customer review.	

Publications

Natalia Reglero-Real, Lorena Pérez-Gutiérrez, Azumi Yoshimura et al. Autophagy modulates endothelial junctions to restrain neutrophil diapedesis during inflammation Immunity 2021-09-14 [PMID: 34363750] (B/N)

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)

Wong M, Ganapathy AS, Suchanec E et al. Intestinal Epithelial Tight Junction Barrier regulation by Autophagy related protein ATG6/beclin 1 Am. J. Physiol., Cell Physiol. 2019-03-20 [PMID: 30892937] (Func)

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] (WB, Human)

Shoji-Kawata S, Sumpter R, Leveno M et al. Identification of a candidate therapeutic autophagy-inducing peptide. Nature. 2013-02-14 [PMID: 23364696] (In Vivo)

Details:
Tat-beclin 1 (L-amino acid) / Tat-Beclin 1 L11 (NBP2-49886) and Tat–beclin 1 (D-amino acid)/Tat-Beclin 1 D11, Retroinverso form (NBP2-49888) along with the control peptide Tat-scrambled (L-amino acid)/Tat-Beclin 1 L11S Peptide, Scrambled Control (NBP2-49887) were tested in-vivo for the induction of autophagy. 6-week-old GFP-LC3 transgenic mice, and normal or CHIKV and WNV Egypt strain virus infected 5-day-old GFP-LC3 mice were injected intra-peritoneally (i.p.) with these Tat-beclin derivative peptides at a dose of 20 mg/Kg body weight (5.3uM/Kg). Brain tissues were analyzed using IHC-Frozen and Western blot analysis for measuring cell death (TUNEL Assay) and p62 expression respectively. Toxicity of these peptides was assessed in 6-day-old C57BL/6J mice via daily injections of Tat-scrambled (L-amino acid, Tat-Beclin 1 L11S) or Tat-beclin 1 (L-amino acid, Tat-Beclin 1 L11) at 15 mg kg-1 and Tat–beclin 1 (D-amino acid, Tat-Beclin 1 D11) at a dose of 20 mg/Kg body weight for 2 weeks (Figure 4).

Procedures

Western Blot protocol for Tat-Beclin 1 Autophagy Inducing Peptide (NBP2-49887)
Tat-Beclin 1 L11S Peptide - Scrambled Control: https://www.novusbio.com/products/tat-beclin-1-peptide_nbp2-49887
WB Protocol for Tat-Beclin 1 L11 or Tat-Beclin 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 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:- http://www.novusbio.com/support/faqs-autophagy-and-lc3
2. Autophagy Research Sub-topics:- http://www.novusbio.com/research-areas/autophagy
3. Support by Application, Protocols:- http://www.novusbio.com/support/support-by-application.html

Immunocytochemistry/Immunofluorescence protocol for Tat-Beclin 1 Autophagy Inducing Peptide (NBP2-49887)

Tat-Beclin 1 L11S Peptide - Scrambled Control: https://www.novusbio.com/products/tat-beclin-1-peptide_nbp2-49887
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 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 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: http://www.novusbio.com/support/faqs-autophagy-and-lc3
2. Autophagy Research Sub-topics: http://www.novusbio.com/research-areas/autophagy
3. Support by Application, Protocols: http://www.novusbio.com/support/support-by-application.html

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Products Related to NBP2-49887

NBP2-49888	Tat-Beclin 1 D11 Autophagy Inducing Peptide - Retroinverso form

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