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Product no AS21 4525

Anti-mNeonGreen | Fluorescent Protein

Product information

Immunogen	KLH-conjugated peptide derived from synthetic peptide, UniProt: A0A1S4NYF2 from common lancelet *Branchiostoma lanceolatum*
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum, in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution add 50 µl, of sterile water.
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	The peptide used to elicit this antibody is conserved in the following expression vectors:

- dCas9-P2A-DHFR(TSc3)-T2A-mNeonGreen [Cloning vector pBM020]
- Cas9-P2A-DHFR(TSc3)-T2A-mNeonGreen [Cloning vector pBM006]
- mNeonGreen4-tDeg [Cloning vector pminiCMV-mNeonGreen4-tDeg]
- ER-localized mNEONGREEN [Binary vector pKT-NM-erNEON]
- mNeonGreen-3xFLAG [Cloning vector pLM160-mNeonGreen]
- mNeonGreen-ty1 [Cloning vector pSAG1-mNeonGreen_TUB1-dTomato]
- mNeonGreen, partial [Binary vector pRATIO2131]

The peptide used to elicit this antibody is not conserved in GFP protein sequence. Antibody is also recognzing mCheery sequence.

Application information

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Recommended dilution	1 µg/1ml (IF), 1 : 1000 - 1 : 5000 (WB)
Expected | apparent MW	Depends upon used fusion partner
Confirmed reactivity	mNG tag in plant (*Arabidopsis thaliana*) and algal vectors (*Chlamydomonas reinhardtii*)
Predicted reactivity	Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known.
Additional information	This antibody is detecting protein sequence of mNG tag in plant and algal vectors.
	This antibody is also reacting to some degree with YFP and mCherry.
Selected references	To be added when available, antibody available in November 2021.
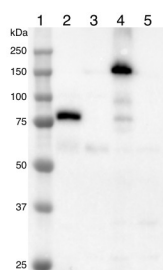
Samples:
1- Precision Plus Protein Standards (Bio-Rad)
2-*Arabidopsis thaliana* expressing mNeonGreen-tagged fusion protein
3-*Arabidopsis thaliana* wildtype total cell extract
4-*Chlamydomonas reinhardtii* expressing mNeonGreen-tagged fusion protein

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5-*Chlamydomonas reinhardtii* wildtype cell extract

20 µg of total protein extracted from Arabidopsis thaliana leaves or Chlamydomonas reinhardtii cells with 62.5 mM Tris-HCl pH 6.8, 10 % glycerol, 2 % SDS, 50 mM DTT, 1x protease inhibitors (P9599, Sigma) and heated at 65ºC for 5 minutes were separated on 10 % SDS-PAGE and blotted 1 h to PVDF membrane (pore size 0.45 µm) using wet transfer. The blot was blocked with 5 % milk/TBS-T for 1 h/RT with agitation and then incubated in the primary antibody at a dilution 1: 1000 in 5 % milk/TBS-T for 1h/RT with agitation. The antibody solution was decanted, and the blot was briefly rinsed, then washed three times for 10 minutes in TBS-T with agitation. The blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG, HRP conjugated, AS09 602) at a dilution of 1: 25 000 in 5 % milk/TBS-T for 1h/RT with agitation. The blot was washed as above, developed for 2 minutes with AgriseraECLSuperBright substrate and imaged with a Bio-Rad ChemiDoc MP digital imaging system. Exposure time was 2.5 s.

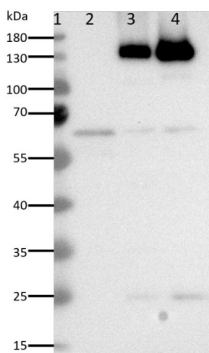Courtesy of Hanie Khorshidi, University of Saskatchewan, Canada
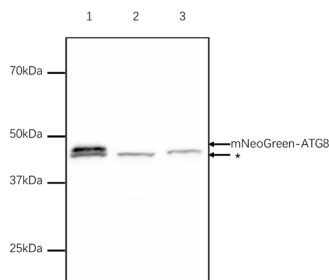
Samples:
1 - *Nicotiana benthamiana* whole leaf extract expressing mNeonGreen-ATG8
2 – *Nicotiana benthamiana* whole leaf extract expressing YFP-ATG8
3 – *Nicotiana benthamiana* whole leaf extract expressing mCherry-ATG8

MW markers: (Biorad)Precision Plus Protein Dual Color Standards, 500 µl #1610374

50 µg of total protein extracted freshly from *Nicotiana benthamiana* leaves with exaction buffer (50mM Tris-HCl pH7.5, 150mM NaCl, 10%(v/v) glycerol, 0.5mM EDTA and 0.5% NP40) denatured with SDS-sample buffer at 95°C for 5 min, proteins were separated on 10 % SDS-PAGE and blotted 0.5h to PVDF membrane using semi-dry transfer. Blots were blocked with 5 % milk in PBS-T for 1h/RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 3 000 for ON/4°C with agitation. The antibody solution was decanted, and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in PBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG (H&L), horse radish peroxidase conjugated, AS09 602) diluted to 1: 5 000 in for 1h/RT with agitation. The blot was washed as above, developed for 5 mins with Agrisera ECL Bright and detected with film.

Courtesy of Dr. Jiaqi Sun, McGill University, Canada

Samples:

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1- PageRuler™ Prestained Protein Ladder (Thermo Fisher)
2- 20 µg *Arabidopsis thaliana* wildtype microsomal membrane preparation
3- 20 µg *Arabidopsis thaliana* expressing VHA-a1-GSL-NeonGreen microsomal membrane preparation
4- 20 µg *Arabidopsis thaliana* expressing VHA-a3-GSL-NeonGreen microsomal membrane preparation

20 µg of microsomal membrane proteins were prepared from *Arabidopsis thaliana* 4-week -old rosette leaves with a buffer containing 350mM sucrose, 70mM Tris-HCl pH8, 10% glycerol, 3mM Na2EDTA, 0.15% BSA, 1.5% PVP-40, 4mM DTT, 1x Roche complete™ protease Inhibitor and denatured at 95ºC for 5 minutes. Proteins were separated on 10 % SDS-PAGE and blotted for 1 h to PVDF membrane (pore size 0.2 µm) using wet transfer. The blot was blocked with 5% milk/TBS-T (0.05%) for 1 h at RT with agitation and then incubated in the primary antibody at a dilution 1: 1000 in 5% milk/TBS-T (0.05%) for 1h at RT with agitation. The antibody solution was decanted, and the blot was briefly rinsed, then washed three times for 10 minutes in TBS-T (0.05%) with agitation. The blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG, HRP conjugated, AS09 602) at a dilution of 1: 25 000 in 5 % milk/TBS-T (0.05%) for 1hr at RT with agitation. The blot was washed as above, developed for 1 min with AgriseraECLBright substrate and imaged with a cooled CCD camera system (Intas ADVANCED Fluoreszenz u. ECL Imager). Exposure time was 10s.

Courtesy of Upendo Lupanga, Centre for Organismal Studies, University of Heidelberg, Germany
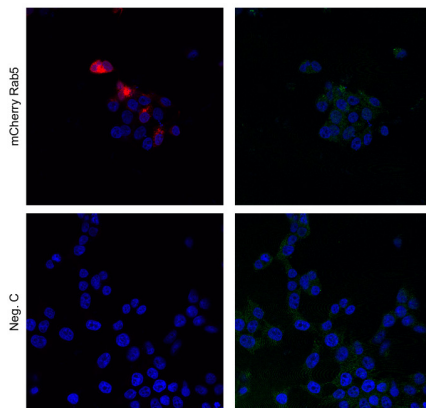
HEK293 cells were transfected with the indicated plasmid (SARS-CoV-2 N myc tagged, PMID: 34799561, TBEV NS3-HA, PMID: 29321318, Rab5 mcherry, Addgene: 4920, GFP-HIS) using genejuice transfection reagent (EMD Millipore) according to the manufacturer's instructions. After 24 hours of transfection, cells were fixed in 4% formaldehyde and permeabilized in PBS containing 0.5% Triton X-100 and 20 mM glycine. Then, cells were stained with the primary anti-mNeonGreen epitope tag, rabbit polyclonal antibodies at a concentration of 1 µg/mL for 1 hour at room temperature. Followed by three washes in PBS. Cells were then stained using secondary antibodies, donkey anti-rabbit Alexa488 (1:500, Thermo Fisher Scientific, a21206 in PBS containing 2% BSA for 1h at RT. Nuclei were stained using DAPI (1 µg/mL). Images were acquired using a Leica SP8 Laser Scanning Confocal Microscope with a 63x oil objective (Leica) and Leica Application Suit X software (LAS X, Leica). Anti-NS3 antibodies are used as a control. Left panel anti-mCheery rab6, right panel protein with mCherry construct, recognized by mNeonGreen antibodies.

Courtesy of Dr. Anna K Överby, Molecular Infection Medicine Sweden (MIMS),Section of Virology. Department of Clinical Microbiology Umeå University, Sweden