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Product no AS12 2220

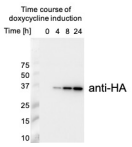
Anti-HA (rabbit polyclonal)

Product information

Immunogen	KLH-conjugated synthetic peptide: YPYDVPDYA
Host	Rabbit
Clonality	Polyclonal
Purity	affinity purified serum in PBS, pH 7.4.
Format	Lyophilized
Quantity	100 µg
Reconstitution	For reconstitution add 100 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.

Application information

Recommended dilution	1 : 5000 (WB)
Expected | apparent MW	Depends on the MW of the protein which is HA-tagged
Confirmed reactivity	HA-tag
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Important note: there is a **strong** cross reactivity between 25 -20 kDa in *Arabidopsis thaliana* wildtype.
Selected references	Tanigawa et al. (2024). FYVE1/FREE1 is involved in glutamine-responsive TORC1 activation in plants. iScience. 2024 Aug 26;27(9):110814. doi: 10.1016/j.isci.2024.110814. Hwang et al. (2019) Arabidopsis ABF3 and ABF4 Transcription Factors Act with the NF-YC Complex to Regulate SOC1 Expression and Mediate Drought-Accelerated Flowering. Mol Plant. 2019 Apr 1;12(4):489-505. doi: 10.1016/j.molp.2019.01.002



10 µg of total protein extracted freshly from mutant HEK293 cells with RIPA buffer and denatured at 65°C for 5 min were separated on 15% SDS-PAGE and blotted overnight to PVDF (pore size of 0.4 um), using wet transfer. Blot was blocked with 5% milk or 2 h at RT and then incubated in the primary antibody at a dilution of 1: 5 000 in a rotator for 2.5 h in 5% milk. The membrane was then placed in a clean container and rinsed 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, AS09 602) diluted to 1:20 000 in for 1 h at RT with agitation. The blot was washed as above and developed with Agrisera ECLSuperBright. Exposure time was 30 seconds.

Courtesy Dr. Małgorzata Wessels, Department of Medical Biochemistry and Biophysics, Umeå University, Sweden

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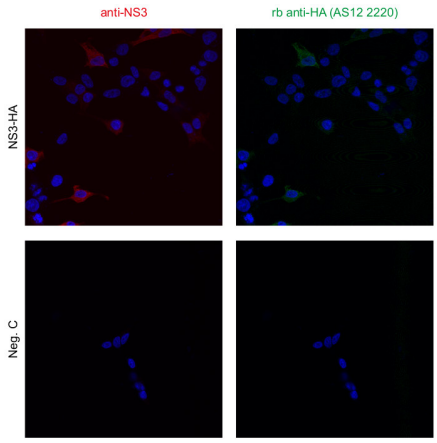

HEK293 cells were transfected with the indicated plasmid (SARS-CoV-2 N myc tagged, PMID: 34799561, TBEV NS3-HA, PMID: 29321318, Rab5 mcherry, Addgene: 4920, GFP-HIS) using genejuice transfection reagent (EMD Millipore) according to the manufacturer's instructions. After 24 hours of transfection, cells 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-HA 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 antirabbit 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.

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