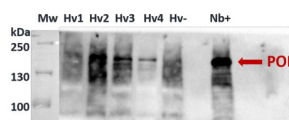


Product no **AS16 3690****Cas9 | CRISPR-associated endonuclease 9 (polyclonal)****Product information**

<b>Immunogen</b>	KLH-conjugated synthetic peptide derived from <i>Streptococcus thermophilus</i> Cas9/Csn1 protein sequence, UniProt: <a href="#">Q03J16</a> (peptide sequence is conserved in pYLCRISPR/Cas9Pubi-H vector)
<b>Host</b>	Rabbit
<b>Clonality</b>	Polyclonal
<b>Purity</b>	Immunogen affinity purified serum in PBS pH 7.4.
<b>Format</b>	Lyophilized
<b>Quantity</b>	50 µg
<b>Reconstitution</b>	For reconstitution add 50 µl of sterile water
<b>Storage</b>	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**

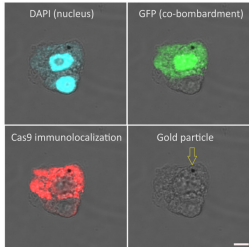
<b>Recommended dilution</b>	1 : 100 (IL), 1 : 1000 (WB)
<b>Expected   apparent MW</b>	Depends upon a MW of a protein which is a fusion partner,
<b>Confirmed reactivity</b>	<i>Staphylococcus aureus</i>
<b>Predicted reactivity</b>	<i>Staphylococcus pyogenes</i> UniProt: <a href="#">Q99ZW2</a>

**Application example**

*Nicotiana benthamiana* (Nb+): sample from 5 weeks old plants second leaves (previously infiltrated with *A.tumefaciens* containing Cas9 transformation cassettes(Nb+). *Hordeum vulgare* (Hv 1-4 , Hv- samples): 2 months old plants' youngest leaves (stably transformed (Hv 1, -2, -3, -4) and wild type (Hv-) plants). 100 mg plant tissue (homogenised in liquid N2) +400 µl extraction buffer (15 min 4 °C 1500rpm centrifugation). Tissue was extracted with following extraction buffer: 10 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl, 10 V% glicerol, 0,5 V% Nonidet P-40, 5 mM NaF. Right before use: 1 mM DTT, 100 mM PMSF, 200 mM Na3VO4 is added to the extraction buffer. Denaturation: sample in extraction buffer + equal volume 2X Laemmli buffer, 72 °C, 10 min.

20 µl of the samples were separated on 8% SDS-PAGE, and blotted with transblot turblo turbo (BioRad) to 0.45 µm pore size PVDF (Hybond, GE). Blots were blocked with 5% milk powder in PBST for 60 min at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a dilution of 1 : 1000 for 160 min at RT with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 2 times for 10 min in PBST buffer at RT with agitation. Blot was incubated in secondary antibody (Goat anti-rabbit from Agrisera, [AS09 602](#)) diluted to 1:20 000 in PBST for 90 min at RT with agitation. The blot was washed as above and developed for 1 min with Bio-Rad Clarity Western ECL substrate using Bio-Rad ChemiDoc™ Imaging System. Exposure time was 170.6 sec.

Courtesy Dr. Éva Hamar, ABC, Hungary



Immunolocalization of Cas9 on maize (*Zea mays*, cv. H1233) plant suspension cultures. Rabbit anti-Cas9 antibody (Agrisera AS16 3690), diluted 1:100 and DyLight 550 anti-rabbit secondary antibody (Agrisera AS11 1782) diluted 1:300, (red) are used for immunodetection following formaldehyde fixation, cell wall digestion and detergent permeabilization of cells. DAPI is used as nuclear marker (blue). Gold particle used for microprojectile bombardment of plasmids (GFP and Cas9) is indicated with an arrow at the last column. Scalebar is 5µm.

Courtesy of Dr. Ferhan Ayaydin, Hungarian Academy of Science, Hungary

### Labeling and detection protocol

Fixation (30 min) 4% paraformaldehyde in PBS (pH 7.4) with 0.01% TritonX-100. (3x5min PBS wash)

Cell wall digestion (40 min): 1% Cellulase, 1% Pectinase in 0.5% (w/v) MES buffer (pH 5.6) (2x5 min PBS wash)

Immobilization of cells (10mins): Cells in PBS were settled onto poly-L-Lysine coated coverslips, excess PBS removed without air drying the cells.

Membrane permeabilization (10 min): 0.5% TritonX-100 in PBS (3x5min PBS wash) Blocking (10mins): 5% Fish gelatin in PBS

Primary antibody incubation (2.5 h at 24 °C): Agrisera (AS16 3690) rabbit anti-Cas9 polyclonal antibody diluted 1:100 in blocking buffer (4x5 min blocking buffer wash)

Secondary antibody incubation (1h at 24 °C): goat anti-rabbit DyLight 550 antibody (Agrisera [AS11 1782](#)) diluted 1:300 in blocking buffer. (3x5min PBS wash)

Nuclear counterstaining (5 min): 200ng/ml DAPI in PBS (brief PBS wash)

Mounting: Fluoromount G mounting medium was used to mount coverslips onto glass slides. Imaging: Olympus FV1000 confocal microscope with 40x (NA1.3) oil immersion objective.