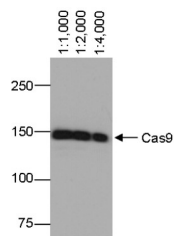


Product no **AS17 4124****Anti-Cas9 | CRISPR-associated endonuclease 9 (monoclonal)****Product information**

<b>Immunogen</b>	Recombinant protein part from the N-terminus of Cas9 from <i>Streptococcus pyogenes</i> .
<b>Host</b>	Mouse
<b>Clonality</b>	Monoclonal
<b>Subclass/isotype</b>	IgG1k
<b>Purity</b>	Total IgG. Protein A purified in PBS. Contains 0.02 % sodium azide.
<b>Format</b>	Liquid
<b>Quantity</b>	50 µg
<b>Storage</b>	Store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. For long term storage -80 °C is recommended. 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.

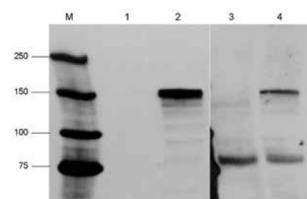
**Application information**

<b>Recommended dilution</b>	1 : 200 (IF), (IP), 1 : 1000-1 : 5000 (WB)
<b>Expected   apparent MW</b>	Depends upon a MW of a protein which is a fusion partner
<b>Confirmed reactivity</b>	Cas9 from <i>Streptococcus pyogenes</i>

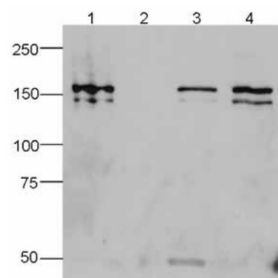
**Application example**

Protein extracts from HEK293 cells were transfected with a myc-tagged Cas9. Visualization was made using monoclonal anti-Cas9 antibodies used in annotated dilutions.

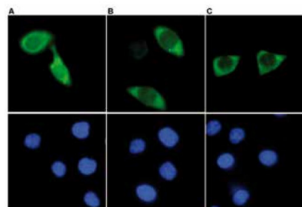
Protein samples were separated on 7.5 % SDS-PAGE and blotted 1h to nitrocellulose membrane. Blots were blocked with 3 % non-fat dry milk in PBS+Tween for 1h at room temperature (RT) with agitation. Blot was incubated in the primary antibody at a given dilution for 1h at RT with agitation in PBS-T. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 5 min in TBS-T at RT with agitation. Blot was incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:10 000 in for 1h at RT with agitation. The blot was washed as above and incubated in ECL solution followed by exposure to an X-ray film.



Protein extracts from HEK293 cells were transfected with a myc-tagged Cas9. Transfected cells (lane 2 and 4) and untransfected cells (lane 1 and 3) cells. Detection using anti-myc antibody (lane 3 and 4).



IP was performed on whole cell extracts (100 µg) from HEK293 cells transfected with a Flag-tagged Cas9 using monoclonal anti-Cas9 antibodies. The immunoprecipitated proteins were subsequently analysed by Western blot with the antibody. Lane 3 and 4 show the result of the IP; a negative IP control (IP on untransfected cells) and the input (15 µg) are shown in lane 2 and 1, respectively.



HeLa cells were transiently transfected with a Flag-tagged Cas9 expression vector. 48 hours post transfection the cells were fixed in 3.7% formaldehyde, permeabilized in 0.5% Triton-X-100 and blocked in PBS containing 2% BSA for 2 hours at RT. The cells were stained with monoclonal anti-Cas9 (A and B) or with an anti-Flag (C) antibody at 4°C O/N, followed by incubation with an anti-mouse secondary antibody coupled to AF488 for 1 h at RT. Nuclei were counter-stained with Hoechst 33342 (bottom).