NEUROMICS

Cas9 from *Staphylococcus aureus* Data Sheet

Catalog Number:	CH22120	**Host:**	Chicken
Product Type:	Polyclonal IgY IgY preparation Antibody	**Species Reactivity:**	Cas9 from *Staphylococcus aureus*
Immunogen Sequence:	C-terminal region of *S. aureus*, amino acids 803-1053 of sequence CCK74173, expressed in and purified from *E. coli*.	**Format:**	Liquid, 100 ul aliquot
Applications:	Western Blot: 1:1,000 Immunofluorescence/Immunocytochemistry: 1:1,000-1:5,000 Immunohistochemistry: 1:1,000-1:5,000 Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Stable at 4°C for one year. Mix antibody 1:1 with 100% glycerol and stored frozen at -20° C for longer term storage		

Application Notes

Description/Data:

The discovery of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has changed the field of gene editing. These repeated sequences are found in bacterial genomes with short DNA sequences derived from viruses which have infected the bacteria interspaced. These virally derived sequences can make short RNA sequences which can hybridize with specific viral DNA and target a nuclease, such as Cas9, to the viral sequence. So, if the bacteria are infected by this virus again, Cas9 can be directed to cleave the specific viral sequence and so inactivate the virus. By careful design of the RNA sequence the system can be used to specifically cut DNA virtually anywhere, including in living human and other mammalian cells. This allows inexpensive gene editing with unprecedented ease, and much effort is going into refining the Cas9 enzymes and their relatives for use in mammalian systems.

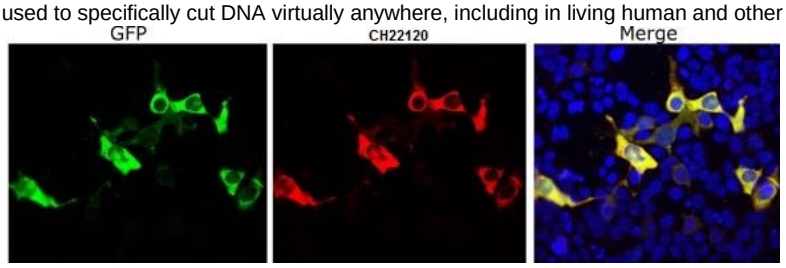

Image: Transfected HEK293 cells which overexpressing GFP-Cas9-SA fusion protein were stained with CH22120. Cells which are transfected with GFP-Cas9 are bright green (left panel). Staining with CH22120 is shown in red in middle panel. In merged image (right panel), most Hek293 cells are not transfected so only the nucleus of these cells can be visualized with a blue DNA stain. Red antibody staining is only seen in cells which express GFP, as expected, and the superimposition of green and red results in an orange signal.

Several varieties of Cas9 have been studied and there appear to be several other related enzymes with similar properties in bacteria. Two Cas9 homologs include *Streptococcus pyogense* and *Staphylococcus aureus*. *Staphylococcus aureus* is significantly smaller and so presents less problems when packaged into vectors. The *S. pyogenes* protein is rather large at 1,368 amino acids, ~160kDa, so the corresponding DNA is also rather large at about 4.2 kb. Our antibody is a polyclonal raised in chicken against the C-terminal 251 amino acids of of the *Staphylococcus aureus* protein and binds this protein transfected into cells, on western blots and in immunocytochemistry. The homologous region of the *S. pyogenes* is not closely related in amino acid sequence and, as expected, this antibody does not recognize that protein.

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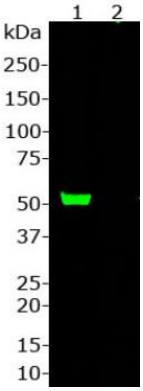

Image: Western blot analysis of CH22120
1: HEK293 cells overexpressing a fusion protein containing GFP and the C-terminus of Cas9 from S. aureus.
2: Non-transfected HEK293 cells.
The band at about 53 kDa corresponds to GFP-Cas9 fusion protein and it is absent from non-transfected cells. CH22120 is at 1: 1,000 dilution

Immunostaining of cells in tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains, generally the most chemically reactive ones like amines (Lysine, Arginine, Glutamine and Asparagine). This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methanol are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most of the widely used human and rodent cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to miss the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps missing out that step. The following procedure works for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well tissue culture plates or in 35mm tissue culture dishes. These are just big enough that you can look from above with a typical immunofluorescence microscope through a glass coverslip. This allows you to see the specimens very well and take very high quality pictures. (However note that it's a pain to changes lenses on the microscope if you use the 6 well dishes, since you have to rack the lens right the way up to do this, and you have to take out the two neighboring lenses in the turret since they will hit the other wells of the dish! It's less of a problem with 35mm dishes but still a pain. No procedure is perfect….).

1. Draw of culture medium with aspirator and add 1 mL of 3.7 % formalin in PBS solution to the dish. (make up from 10 mLs Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).

2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well-sealed bottle in fridge). Let sit for no more than 1 minute.

3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10 µL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100 µL of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).

4. Remove primary antibody and replace with 1 mL of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

5. Add 0.5 µLs of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and were originally marketed by Molecular Probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc. Molecular Probes was bought by Invitrogen, which now markets these reagents). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).

6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

7. Drop on one drop of Fisher mounting medium onto dish and apply 22 mm square coverslip. View in the microscope!

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