abcam

**ab204448**

# Anti-CRISPR-Cas9 antibody

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | Preservative: 0.05% Sodium azide |
| **Form** | Liquid |
| **Clonality** | Polyclonal |
| **Immunogen** | Recombinant Fragment Protein within Streptococcus pyogenes serotype M1 cas9. The exact immunogen used to generate this antibody is proprietary information. Database link Q99ZW2 |
| **Purity** | Whole antiserum |
| **Specificity** | This antibody reacts with CRISPR-Cas9, this antibody has been found to react with 6xHis and Flag tags. If using multiple tags we recommend ab189380. |

## Reactivity data

## IP

### Tested

| | |
|---|---|
| **Species** | Transfected cell lysate - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | Use 10µl per IP. |

### Predicted

| | |
|---|---|
| **Species** | Streptococcus pyogenes |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Transfected cell line - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Transfected cell line - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Transfected cell lysate - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Transfected cell lysate - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Transfected cell line - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Transfected cell line - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Transfected cell lysate - Streptococcus pyogenes |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information cas9

| | |
|---|---|
| **Function** | CRISPR (clustered regularly interspaced short palindromic repeat) is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids) (PubMed:21455174). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and this protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA; Cas9 only stabilizes the pre-crRNA:tracrRNA interaction and has no catalytic function in RNA processing (PubMed:24270795). Subsequently Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer; Cas9 is inactive in the absence of the 2 guide RNAs (gRNA). The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3'-5' exonucleolytically. DNA-binding requires protein and both gRNAs, as does nuclease activity. Cas9 recognizes the protospacer adjacent motif (PAM) in the CRISPR repeat sequences to help distinguish self versus nonself, as targets within the bacterial CRISPR locus do not have PAMs. DNA strand separation and heteroduplex formation starts at PAM sites; PAM recognition is required for catalytic activity (PubMed:24476820). Confers immunity against a plasmid with homology to the appropriate CRISPR spacer sequences (CRISPR interference) (PubMed:21455174). |

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

Older lots react with cas9 and the Flag and 6x His tags.
New lots react with only cas9 and the 6x His tag.

Please contact our scientific support team for clarification.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | CRISPR-Cas9 also known simply as Cas9 is a protein that acts as a molecular scissor in gene editing. It has a molecular weight of approximately 160 kDa. Cas9 is a part of the CRISPR-Cas system originally discovered in bacteria where it serves as an adaptive immune system. In bacterial cells Cas9 targets and cleaves specific DNA sequences allowing for targeted gene modifications. While the expression of the CRISPR-Cas system occurs naturally in prokaryotes scientists now harness it in various organisms for genetic manipulation. |
| **Biological function summary** | The Cas9 protein functions as an integral part of the CRISPR-Cas9 complex which includes a guide RNA to direct the protein to specific DNA sequences. This complex enables precise cuts at targeted locations within the genome. The Cas9 protein size allows it to fit effectively within cells facilitating genome editing in areas such as research agriculture and therapeutics. The complexity of CRISPR-Cas9 also includes the interaction with other cellular components that assist in DNA repair post-cleavage. |
| **Pathways** | CRISPR-Cas9 plays a role in DNA repair pathways particularly non-homologous end joining and homologous recombination. After Cas9-induced DNA breaks these pathways become active to repair the cleaved DNA. The gene editing process facilitated by CRISPR-Cas9 often involves interaction with DNA repair proteins like ku70/80 and Rad51 in response to induced breaks. This allows for either the incorporation of new genetic material or the modification of existing genes. |
| **Associated diseases and disorders** | CRISPR-Cas9 has potential in treating genetic disorders such as cystic fibrosis and Duchenne muscular dystrophy. By correcting mutated genes scientists aim to restore normal gene function offering potential therapeutic benefits. Additionally CRISPR-Cas9 relates indirectly to p53 a protein known for its tumor suppressor functions because gene-editing processes can sometimes activate p53-dependent pathways leading to cell cycle arrest or apoptosis. This highlights the importance of understanding the broader implications of CRISPR-Cas9 in therapeutic applications. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

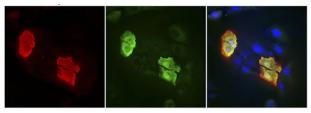
We do not recommend this combination. It is not covered by our product promise.

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In the unlikely event of one of our products not working as expected, you are covered by our product promise.

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## 3 product images

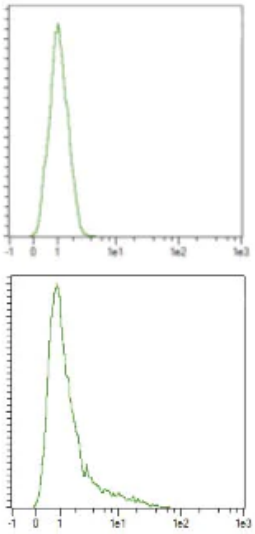


### Immunocytochemistry/ Immunofluorescence - Anti-CRISPR-Cas9 antibody (ab204448)

CRISPR-Cas9 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-CRISPR-Cas9 antibody

Immunofluorescent analysis of U-2 OS (Human bone osteosarcoma epithelial cell line) cells transfected with DDDDK tag-CRISPR-Cas9, labeling CRISPR-Cas9 with ab204448 at 1/10,000 dilution (green). Labeling with anti-DDDDK tag (red). Right hand panel shows merged image with DAPI staining (blue).
Protocol: Fixation with PFA 2% 10 min, permeabilization cold methanol, block with BSA 2% for 1h at RT, incubation with ab204448 1/10,000 1h 30 min at RT.

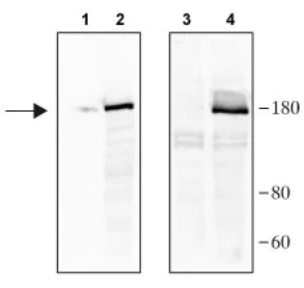
There is a low background staining with ab204448 which is minimized at 1/10000 dilution.



### Flow Cytometry - Anti-CRISPR-Cas9 antibody (ab204448)

CRISPR-Cas9 Flow Cytometry staining using rabbit Anti-CRISPR-Cas9 antibody

Flow cytometric analysis of HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cells (upper panel), and transfected with CRISPR-Cas9 (lower panel) labeling CRISPR-Cas9 with ab204448 at 1/5000 dilution.
Cells were fixed with 1% PFA and incubated with the primary antibody (1/5000) at RT for 2 hours.




### Western blot - Anti-CRISPR-Cas9 antibody (ab204448)

WB: Transfer in nitrocelullose, block: 1 hour 5% milk in TBST (Tween= 0.2%),
Incubation with primary antibody o/n at 4°C in 5% milk TBST (Tween= 0.2%).

Lanes 1 - 2:
Anti-DDDDK tag antibody

Lanes 3 - 4:
Western blot - Anti-CRISPR-Cas9 antibody (ab204448) at 1/2000 dilution

Lane 1:
HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate at 40 µg

Lanes 2 and 4:
HEK-293T cells transfected with DDDDK tag-CRISPR-Cas9 lysate at 40 µg

Lane 3:
HEK-293T cell lysate at 40 µg

Predicted band size: 158 kDa

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Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.