For Research Use Only.
Not for use in diagnostic procedures.

Anti-SaCas9 mAb

CODE No.	D366-3
CLONALITY	Monoclonal
CLONE	11E7-9
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Recombinant protein, corresponding to amino acids 1-462 of *Staphylococcus aureus* Cas9
REACTIVITY	This clone specifically reacts with *Staphylococcus aureus* Cas9 (SaCas9) and does not cross-reacts with *Streptococcus pyogens* Cas9 (SpCas9).
FORMULATION	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Western blotting	0.5 µg/mL
Immunoprecipitation	1 µg/sample
Immunocytochemistry	1 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Staphylococcus aureus
Cell	Not tested	Not tested	Not tested	Transfectant
Reactivity				+

REFERENCE 1) Ran, F. A., *et al., Nature* **520**, 186-191 (2015)

For more information, please visit our website at **https://ruo.mbl.co.jp/**.

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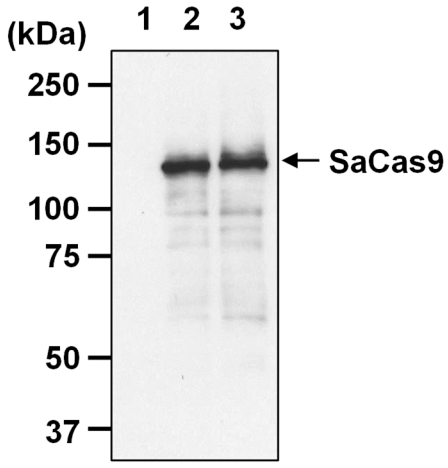
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (3 times for 5 min.).
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film for 1 min. in a dark room. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blotting analysis of SaCas9

Lane 1: 293T
Lane 2: SaCas9-DN-HA/293T
Lane 3: SaCas9-WT-HA/293T

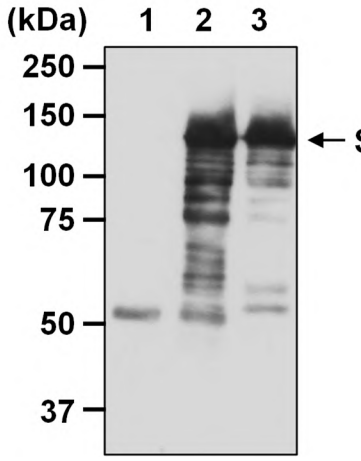
Immunoblotted with Anti-SaCas9 mAb (MBL, code no. D366-3)

Samples were kindly provided by Dr. Haruhiko Siomi.
(Department of Molecular Biology, Keio University School of Medicine)

Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them with 2 mL of Extraction buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, then sonicate briefly (up to 15 sec.).
2) Incubate the tube for 15 min. on ice.
3) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 200 µL of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the beads with 1 mL of IP buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 6)-7) twice.
9) Add 200 µL of cell lysate (prepared sample from step 3) into the tube. Incubate with gentle agitation for 1 hr. at room temperature.
10) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
11) Resuspend the beads with 1 mL of Extraction buffer.
12) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
13) Repeat steps 11)-12) 5 times.
14) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
15) Load 10 µL per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
16) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
17) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) overnight at 4°C.
18) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
19) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (3 times for 5 min.).
20) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS) for 1 hr. at room temperature.
21) Wash the membrane with PBS-T (3 times for 5 min.).
22) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
23) Expose to an X-ray film for 1 min. in a dark room. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Transfectant)



Immunoprecipitation of SaCas9 from 293T transfectant
Sample: SaCas9-WT-HA/293T
Lane 1: Mouse IgG2a (isotype control) (MBL, code no. M076-3)
Lane 2: Anti-SaCas9 mAb (MBL, code no. D366-3)
Lane 3: Anti-HA-tag mAb (MBL, code no. M180-3)

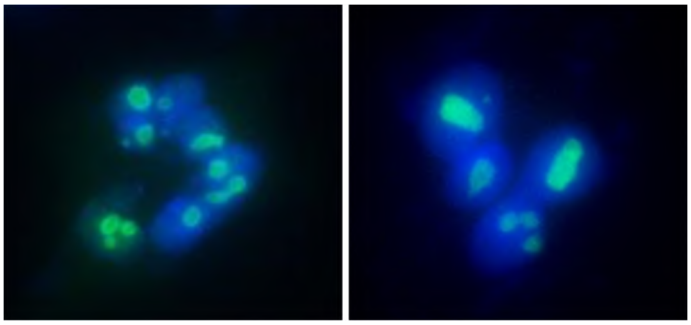
Immunoblotted with Anti-SaCas9 mAb (MBL, code no. D366-3)

Sample was kindly provided by Dr. Haruhiko Siomi.
(Department of Molecular Biology, Keio University School of Medicine)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide twice with PBS.
4) Fix the cells with 4% paraformaldehyde/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide twice with PBS.
6) Permeabilize the cells with 0.1% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide twice with PBS.
8) Tip off PBS and add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 3 times with PBS.
10) Add 200 µL of 1:500 Alexa Fluor® 488 Goat Anti-mouse IgG (Thermo Fisher Scientific, code no. A-11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 3 times with PBS.
12) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
13) Counterstain with DAPI for 5 min. at room temperature.
14) Wash the slide once with PBS.
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Transfectant)



Immunocytochemical detection of SaCas9 in HeLa transfectants

Right: SaCas9-WT-HA/HeLa
Left: SaCas9-DN-HA/HeLa
Green: Anti-SaCas9 mAb (MBL, code no. D366-3)
Blue: DAPI

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(Department of Molecular Biology, Keio University School of Medicine)