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

Anti-SaCas9 mAb-HRP-DirecT

CODE No. D366-7

CLONALITY Monoclonal
CLONE 11E7-9
ISOTYPE Mouse IgG2a κ
QUANTITY 50 μL

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).
FORMURATION PBS containing 1% BSA and 0.1%ProClin 150
STORAGE This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

Western blotting 1:10,000 for chemiluminescence detection system

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 web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
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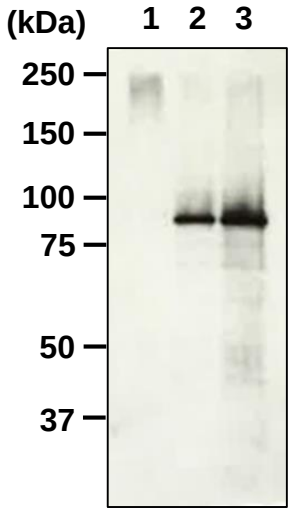
RELATED PRODUCT

D366-3 Ant-SaCas9 mAb (11E7-9)

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% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS) as suggested in the **APPLICATION** 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) 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.
8) 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 blot analysis of SaCas9

Lane 1: 293T (1000 cells/lane)
Lane 2: SaCas9-DN-HA/293T (25 cells/lane)
Lane 3: SaCas9-WT-HA/293T(100 cells/lane)

Immunoblotted with Anti-SaCas9 mAb-HRP-DirecT (D366-7)

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