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

Fast Line Product Series

Anti-RORγt mAb

CODE No. M219-3

CLONALITY Monoclonal
CLONE 4H11
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from mouse ascites fluid
IMMUNOGEN KLH-conjugated synthetic peptide, corresponding to N-terminal region of human RORγt (This region shares 100% amino acid sequence homology with that of mouse RORγt.)
FORMURATION 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	1 µg/mL for chemiluminescence detection system
Immunoprecipitation	2 µg/sample

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Transfectant	Not tested*	Not tested	Not tested
Reactivity	+			

*The reactivity to mouse is under evaluation.

Entrez Gene ID 6097 (Human)

For more information, please visit our web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

M219-3	Anti-RORγt mAb
PM080	Anti-RORγt pAb
D222-3	Anti-GITR (Mouse) mAb
D222-4	Anti-GITR (Mouse) mAb-FITC
D222-5	Anti-GITR (Mouse) mAb-PE
D239-3M2	Anti-FR4 (Mouse) mAb (Functional grade)
D239-3	Anti-FR4 (Mouse) mAb
D239-4	Anti-FR4 (Mouse) mAb-FITC
D239-5	Anti-FR4 (Mouse) mAb-PE
D239-6	Anti-FR4 (Mouse) mAb-Biotin
PM024	Anti-Foxp3 pAb
D237-4	Anti-Foxp3 (Mouse) mAb-FITC
D237-6	Anti-Foxp3 (Mouse) mAb-Biotin
M120-3	Anti-Foxp3 mAb
D091-3	Anti-CTLA-4 (CD152) mAb
D091-6	Anti-CTLA-4 (CD152) mAb-Biotin
PM081	Anti-Cry1 (Mouse) pAb
PM082	Anti-Cry2 (Mouse) pAb
PM083	Anti-Per2 (Mouse) pAb
D333-3	Anti-CLOCK (Mouse) mAb (CLSP3)
D334-3	Anti-CLOCK (Mouse) mAb (CLNT1)
D335-3	Anti-BMAL1 (Mouse) mAb (B1BH2)
D349-3	Anti-CLOCK (Mouse) mAb (CLSP4)
PM079	Anti-DBP (Mouse) pAb
CY-P1016	Anti-SIRT1 pAb
RN032P	Anti-CIRBP pAb
PM075	Anti-GNAT2 (Zebrafish) pAb

Kits

CY-1151	CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit
CY-1152	CycLex® SIRT2 Deacetylase Fluorometric Assay Kit
CY-1173	CycLex® CaM-kinase II Assay Kit
CY-1252	CycLex® NMNAT Colorimetric Assay Kit

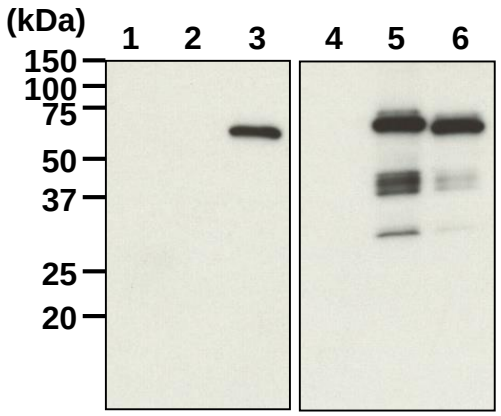
Recombinant proteins (Human, Active)

CY-E1151	NAD^+-Dependent Deacetylase SIRT1
CY-E1152	NAD^+-Dependent Deacetylase SIRT2
CY-E1173	CaM-kinase II Positive Control
CY-E1251	NAMPT (Human, Active)

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL Laemmli's sample buffer.
2) Boil the sample for 5 min. and centrifuge. Load 1 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% 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, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 Goat anti-Rat IgG Antibody, HRP conjugate (Millipore; code no. AP136P) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) Wipe excess buffer on the membrane, 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 a plastic wrap.
11) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blot analysis of human RORγt

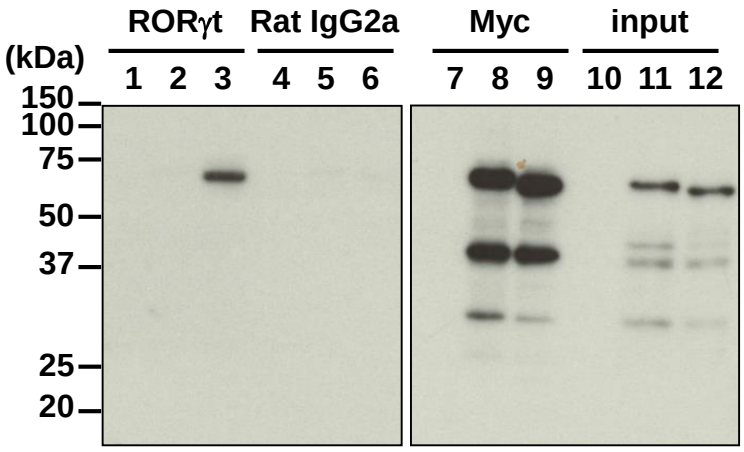
Lane 1, 4: HEK293T
Lane 2, 5: Myc-tagged RORγ/HEK293T
Lane 3, 6: Myc-tagged RORγt/HEK293T

Immunoblot
Lane 1-3: Anti-RORγt mAb (M219-3)
Lane 4-6: Anti-Myc-tag mAb (M192-3)

Immunoprecipitation

1) Wash 2 x 10^7 cells 3 times with PBS and resuspend them in 3 mL of Extraction buffer [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add 100 µL of 50% protein G agarose beads slurry resuspended in PBS-T [0.05% Tween-20 in PBS] into the 300 µL of the supernatant. Incubate it at 4°C with rotating for 1 hr.
4) Centrifuge the tube and transfer the supernatant to another tube (precleared sample).
5) Add 70 µL of 50% protein G agarose beads slurry resuspended in 300 µL of PBS-T with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation overnight at 4°C.
6) Wash the beads 4 times with 1 mL of PBS-T.
7) Add 300 µL of the precleared sample (prepared in step 4)) to the tube containing antibody conjugated beads, then incubate with gentle agitation overnight at 4°C.
8) Wash the beads 4 times with 1 mL of PBS-T.
9) Resuspend the beads in 50 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
10) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
11) 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.
12) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
13) Wash the membrane with PBS-T (5 min. x 3 times).
14) Incubate the membrane with 1:2,000 of Anti-Myc-tag mAb-HRP DirecT (MBL; code no. M047-7) diluted with 1:100 of Clear Back for IP Western (MBL; code no. MTG-002) in 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
15) Wash the membrane with PBS-T (5 min. x 3 times).
16) Wipe excess buffer on the membrane, 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 a plastic wrap.
17) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Transfectant)



Immunoprecipitation of human RORγt from transfectant

<Sample>
Lane 1, 4, 7, 10: HEK293T
Lane 2, 5, 8, 11: Myc-tagged RORγ/HEK293T
Lane 3, 6, 9, 12: Myc-tagged RORγt/HEK293T

Lane 1-3: Anti-RORγt mAb (M219-3)
Lane 4-6: Rat IgG2a (isotype control) (M081-3)
Lane 7-9: Anti-Myc-tag mAb (M192-3)
Lane 10-12: Input (total cell lysate)

Immunoblotted with Anti-Myc-tag mAb -HRP-DirecT (M047-7)