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

MONOCLONAL ANTIBODY

Anti-RUNX3 mAb

Code No.	Clone	Subclass	Quantity	Concentration
D234-3	R3-6E9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: The Runx (runt-related protein) family of transcription factors plays important roles in different tissues and cell lineages. Runx1 determines commitment to the hematopoietic cell lineage and Runx2 determines commitment to the osteoblastic lineage. Runx3 is involved in gastric epithelial growth and differentiation. PEBP2/Cbfβ is required for Runx-dependent transcriptional regulation. Runx proteins interact with many other transcription factors and co-regulators in the transcriptional regulation of their target genes.

SOURCE: This antibody was purified from hybridoma (clone R3-6E9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with human RUNX3 polypeptide antigens.

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with human RUNX3 (hRUNX3) and mouse Runx3 (mRunx3) (191-300 aa), on Western blotting, and reacts with hRUNX3 on Immunohistochemistry. Clone R3-6E9 doesn't react with human and mouse Runx1/2.

APPLICATIONS:

Western blotting; 0.2-1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1).
Immunohistochemistry; 2 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Autoclave; 105°C for 15 min in antigen retrieval solution.
Immunocytochemistry; Not tested
Flow cytometry; Not tested
Chromatin Immunoprecipitation; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2), 3) and 5).

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Samples	Recombinant	Recombinant	Not tested
Reactivity on WB	+	+	

Species	Human	Mouse	Rat
Reactivity on IHC	+	-	Not tested

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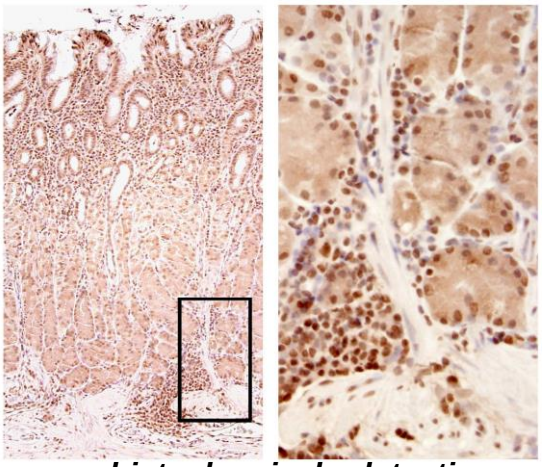
1) Qiao, Y., *et al.*, *Oncogene* **35**, 2664-2674 (2016) [IP]
2) Hor, Y. T., *et al.*, *Cell Rep.* **8**, 50-58 (2014) [ChIP]
3) Presnell, S. R., *et al.*, *J. Immunol.* **188**, 4394-4404 (2012) [ChIP]
4) Lin, F. C., *et al.*, *Oncogene* **31**, 4302-4316 (2012) [IHC]
5) Chang, T. L., *et al.*, *Gastroenterology.* **138**, 255-265 (2010) [IHC, ChIP]
6) Fujii, S., *et al.*, *J. Biol. Chem*. **283**, 17324-17332 (2008)
7) Lau, Q. C., *et al.*, *Cancer Res*. **66**, 6512-6520 (2006)
8) Yano, T., *et al*., *Mol. Cell Biol*. **26**, 4474-4488 (2006)
9) Ito, K., *et al.*, *Cancer Res.* **65**, 7743-7750 (2005)



Immunohistochemical detection of RUNX3 on paraffin embedded section of human stomach with D234-3. RUNX3 is strongly expressed in gastric epithelial cells and lymphocytes.

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PROTOCOLS:

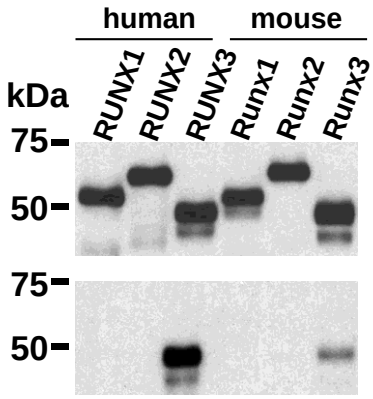
Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Autoclave:
   Autoclave slides in the antigen retrieval solution (DAKO; code no. S1700) for 15minutes at 105°C. Let the slides cool down to room temperature for about 40 minutes.
5) Remove the slides from the antigen retrieval solution and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
8) Incubate the sections at 4°C overnight.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunohistochemistry; human stomach)

SDS-PAGE & Western Blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 as suggested in the **APPLICATIONS** for 1 hour 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] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes.
12) Develop the film under usual settings. The conditions for exposure and development may vary.



Western blot analysis of recombinant Runx.
Upper panel: D207-3 (3D9)
Lower panel: D234-3 (R3-6E9)

The data of WB and IHC were kindly provided from Dr. Yoshiaki Ito and Dr. Kosei Ito. (Institute of Molecular and Cell Biology, Singapore)

RELATED PRODUCTS:

D235-3 Anti-RUNX3 mAb (R3-5G4)
D236-3 Anti-RUNX3 mAb (R3-1E10)
D130-3 Anti-Runx2 (Cbfa1) mAb (8G5)
D207-3 Anti-Runx mAb (3D9)
D208-3 Anti-Runx mAb (6B4)
D127-3 Anti-PEBP2β (CBFβ) (Human) mAb (β122)
M075-3 Mouse IgG1 (isotype control) (2E12)