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

MONOCLONAL ANTIBODY

Anti-Runx

Code No.	Clone	Subclass	Quantity	Concentration
D207-3	3D9	Mouse IgG1	100 µg	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 3D9) supernatant using protein A agarose. This hybridoma was established by Balb/c mouse splenocyte immunized with the recombinant polypeptide of the Runt domain.

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 the Runt domain of human Runx and mouse Runx on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
others	Recombinant	Recombinant	Not Tested
Reactivity on WB	+	+	

INTENDED USE:
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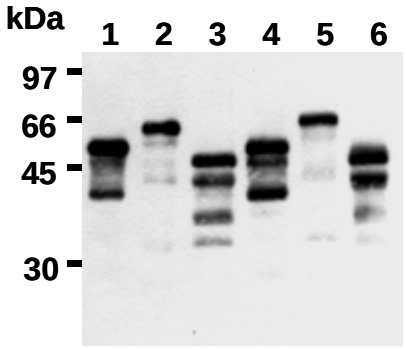
REFERENCE:
1) Ito, K., *et al.*, *Cancer Res.* **65**, 7743-7750 (2005)

Clone 3D9 is used in this reference.

RELATED PRODUCTS:
D130-3 Anti-Runx2/Cbfa1 (8G5)
D208-3 Anti-Runx (6B4)
D127-3 Anti-PEBP2β (β122)



Western blot analysis of human recombinant Runx1 (1), Runx2 (2), Runx3 (3), mouse recombinant Runx1 (4), Runx2 (5) and Runx3 (6) using D207-3.

PROTOCOL:

SDS-PAGE & Western Blotting

1) Mix the recombinant protein with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for 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 manufacture'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 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
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 (5 minutes x 6 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; recombinant human Runx and mouse Runx)