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

MONOCLONAL ANTIBODY

Anti-RUNX3

Code No.	Clone	Subclass	Quantity	Concentration
D235-3	R3-5G4	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 R3-5G4) 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. Clone R3-5G4 doesn't react with human and mouse Runx1/2.

APPLICATIONS:

Western blotting; 0.2 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	recombinant, HGC-27, MKN1, MKN45, OCUM-1, Saos-2	recombinant	Not Tested
Reactivity on WB	+	+	

INTENDED USE:
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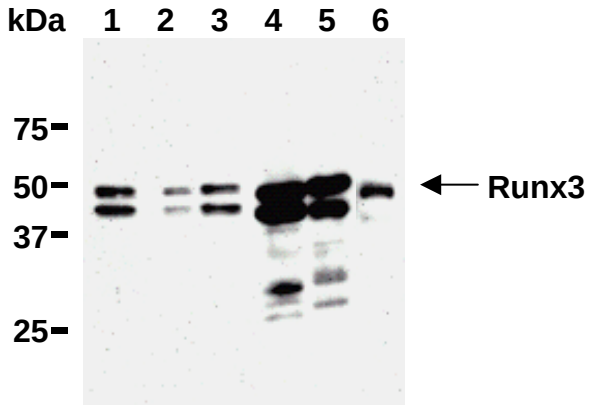
REFERENCES:
1) Yano, T., *et al*., *Mol. Cell Biol*. **26**, 4474-4488 (2006)
2) Ito, K., *et al., Cancer Res.* **65**, 7743-7750 (2005)

Clone R3-5G4 is used in reference number 2).

RELATED PRODUCTS:
D234-3 Anti-RUNX3 (R3-6E9)
D236-3 Anti-RUNX3 (R3-1E10)
D130-3 Anti-Runx2/Cbfa1 (8G5)
D207-3 Anti-Runx (3D9)
D208-3 Anti-Runx (6B4)
D127-3 Anti-PEBP2β (β122)



Western blot analysis of RUNX3 expression in HGC-27 (1), MKN1 (2), MKN45 (3), OCUM-1 (4), Saos-2 (5) and recombinant mRunx3 (6) using D235-3.

PROTOCOL:
SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.

3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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.
6) 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^oC.
7) 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.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) 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.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; recombinant, HGC-27, MKN1, MKN45, OCUM-1, Saos-2)