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MONOCLONAL ANTIBODY

Anti-Runx2 (Cbfa1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D130-3	8G5	Mouse IgG2b κ	100 µL	1 mg/mL

BACKGROUND: Runx2, also known as Cbfa1 or PEBP2αA, is an essential transcription factor of skeletal tissues that is involved in the regulation of osteoblast differentiation and bone formation. Runx2-null mice have neither bone tissue nor osteoblasts. FGF receptor signaling, TGF-β, and BMP all activate transcription of Runx2, resulting in inhibition of myogenesis and myogenic differentiation. BMP-induced Runx2 cooperates with BMP-activated Smads to induce osteogenesis. Runx2 has also been shown to play a role in the regulation of chondrocyte hypertrophy and tooth eruption.

SOURCE: This antibody was purified from hybridoma (clone 8G5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell X63-Ag8.653 with Balb/c mouse splenocyte immunized with the recombinant Runx2.

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 Runx2/Cbfa1 (66 kDa) and mouse Runx2/Cbfa1 (55 kDa) on Western blotting.

APPLICATION-CONFIRMED:
Western blotting; 1 µg/mL

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

APPLICATIONS-REPORTED:
Immunoprecipitation; Reference 8) and 12)
Immunohistochemistry; Reference 1), 3) and 6)
Immunocytochemistry; Reference 4) and 10)
ELISA; Reference 2)
Chromatin Immunoprecipitation; Reference 5), 8) and 9)

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse*	Rat**
Cells	Saos2	2T3, C2C12	Not tested
Reactivity on WB	+	+	

*This monoclonal antibody is widely used to detect mouse Runx2/Cbfa1[5), 7)-9), 11)].
**The cross reactivity to rat Runx2/Cbfa1 is reported[1), 3), 6)].

REFERENCES:
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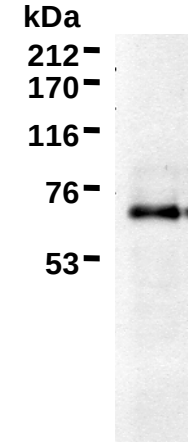
3) Hosoya, A., *et al.*, *J. Histochem. Cytochem.* **60**, 861-73 (2012) [IHC]
4) Ali, S. A., *et al., J. Cell Sci.* **125,** 2732-2739 (2012) [IC]
5) Fulzele, K., *et al., Cell* **142**, 309-319 (2011) [ChIP]
6) Hirata, A., *et al.*, *J. Histochem. Cytochem.* **57**, 397-403 (2009) [IHC]
7) Tominaga, H., *et al.*, *Mol. Biol. Cell* **19**, 5373-5386 (2008) [WB]
8) Ohba, S., *et al., FASEB J.* **21**, 1777-1787 (2007) [WB, IP, ChIP]
9) Wang, Q., *et al., J. Biol. Chem.* **282**, 10742-10748 (2007) [ChIP]
10) Lau, Q. C., *et al.*, *Cancer Res.* **66**, 6512-6520 (2006) [WB, IC]
11) Kaneki, H., *et al.*, *J. Biol. Chem.* **281**, 4326-4333 (2006) [WB]
12) Wee, H. J., *et al.*, *EMBO Rep.* **3**, 967-974 (2002) [WB, IP]
13) Zhang, Y. W., *et al.*, *PNAS* **97**, 10549-10554 (2000) [WB]

As this monoclonal antibody is widely used, many researches have been reported. These references are a part of such reports.



Western blot analysis of Runx2 expression in Saos2 cells using D130-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 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 2 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% methanol). 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°C.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (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 (5 minutes x 6).
11) 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.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Saos2)

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