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

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Anti-Vinculin pAb

CODE No. PM088

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
FORMULATION 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.

APPLICATION-CONFIRMED
Western blotting 1:1,000

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa	NIH/3T3	PC12	CHO
Reactivity	+	+	+	+

Entrez Gene ID 7414 (Human), 22330 (Mouse), 305679 (Rat), 100759958 (Hamster)

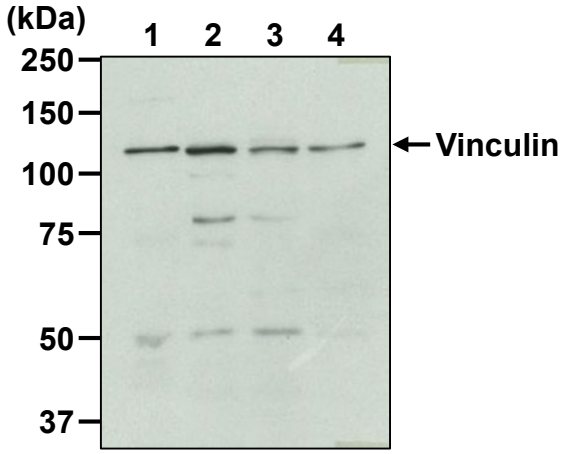
For more information, please visit our web site **https://ruo.mbl.co.jp**.

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

SDS-PAGE & Western blotting

1) Wash cells 3 times with PBS and suspend them in Extraction buffer [50 mM Tris-HCl (pH7.5), 150 mM NaCl, 0.05% NP-40], then sonicate briefly (up to 10 sec.).
2) Centrifuge the tube at 12,000 x g for 10 min. at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant.
3) Add equal volume of 2 x Laemmli's sample buffer and mix well.
4) Boil the sample for 3 min. and centrifuge. Load 10 µg of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
5) 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% methanol). See the manufacturer's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) 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 **APPLICATION** for 1 hr. 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 min. x 3).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
10) Wash the membrane with PBS-T (5 min. x 3).
11) Wipe excess buffer on the membrane, and 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 plastic wrap.
12) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, NIH/3T3, PC12 and CHO)



Western blotting analysis of Vinculin

Lane 1: HeLa
Lane 2: NIH/3T3
Lane 3: PC12
Lane 4: CHO

Immunoblotted with Anti-Vinculin pAb (MBL, code no. PM088)