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

Anti-KEAP1 mAb

CODE No. M224-3

CLONALITY Monoclonal
CLONE KP1
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human KEAP1, recombinant protein
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 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human*	Mouse	Rat	Hamster
sample	HEK293T, A549, HepG2	MEF, NIH/3T3	Rat1, NRK	CHO
Reactivity	+	+	+	+

*This antibody does not react with HeLa cells.

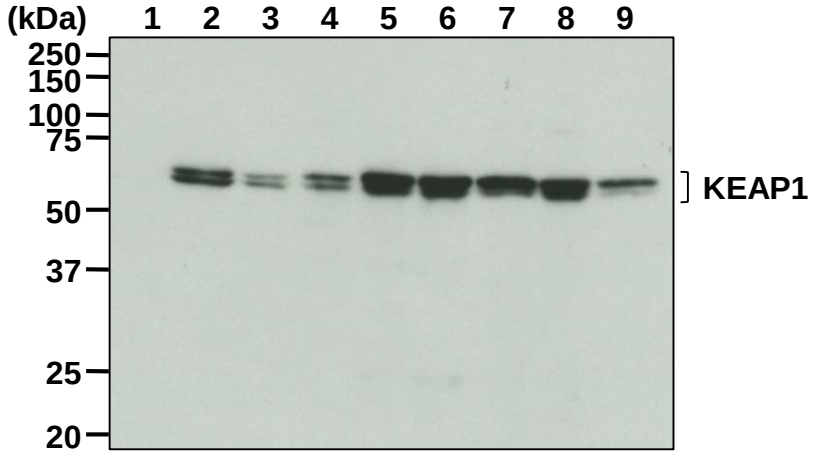
Entrez Gene ID 9817 (Human), 50868 (Mouse), 117519 (Rat), 100759410 (Hamster)

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the sample for 5 min. and centrifuge.
3) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a dry transfer system.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (5 min. x 3).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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, 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 control for Western blotting; HeLa, HEK293, A549, HepG2, MEF, NIH/3T3, Rat1, NRK and CHO)



Western blotting analysis of KEAP1

Lane 1: HeLa
Lane 2: HEK293T
Lane 3: A549
Lane 4: HepG2
Lane 5: MEF
Lane 6: NIH/3T3
Lane 7: Rat1
Lane 8: NRK
Lane 9: CHO

Immunoblotted with Anti-KEAP1 mAb (MBL; code no. M224-3)