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Anti-Parkin mAb

CODE No. M230-3

CLONALITY Monoclonal
CLONE Par6
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human Parkin, full-length (recombinant)
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 for chemiluminescence detection system

SPECIES CROSS REACTIVITY on WB

Species	Human*	Mouse	Rat	Hamster
Samples	Transfectant	Brain lysate	Brain lysate, PC12	Not tested
Reactivity	+	+	+	

*This antibody does not react with HeLa or HEK293T cells.

Entrez Gene ID 5071 (Human), 50873 (Mouse), 56816 (Rat)

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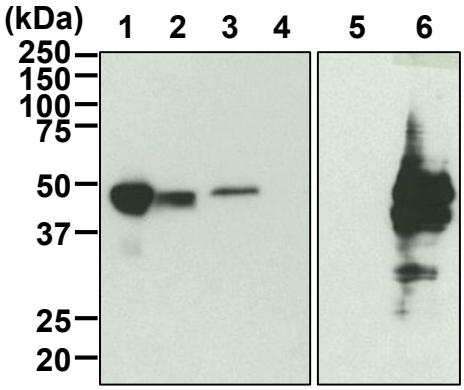
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 20 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 times).
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 (5 min. x 3 times).
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 times).
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 controls for Western blotting; Rat brain, mouse brain, PC12 and transfectant)



Western blot analysis of Parkin
Lane 1: Rat brain lysate, 20 μg
Lane 2: Mouse brain lysate, 20 μg
Lane 3: PC12
Lane 4: HeLa
Lane 5: HEK293T
Lane 6: Human Parkin/HEK293T

Immunoblotted with Anti-Parkin mAb (M230-3)