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

MBL
A JSR Life Sciences Company

Anti-p62 (SQSTM1) (Human) mAb -Alexa Fluor® 647

CODE No. M162-A64

CLONALITY Monoclonal
CLONE 5F2
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Recombinant Human p62 (120-440 a.a.)
FORMULATION PBS containing 1% BSA and 0.1% ProClin 150
STORAGE This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATIONS-CONFIRMED

Immunocytochemistry 5 µg/mL
Flow cytometry 1 µg/mL

SPECIES CROSS REACTIVITY on IC

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant	Not tested	Not tested	Not tested
Reactivity	+			

Entrez Gene ID 8878 (Human)

REFERENCES

1) Ichimura, Y., *et al.*, *J. Biol. Chem.* **283**, 22847-22857 (2008)
2) Komatsu, M., *et al.*, *Cell* **131**, 1149-1163 (2007)

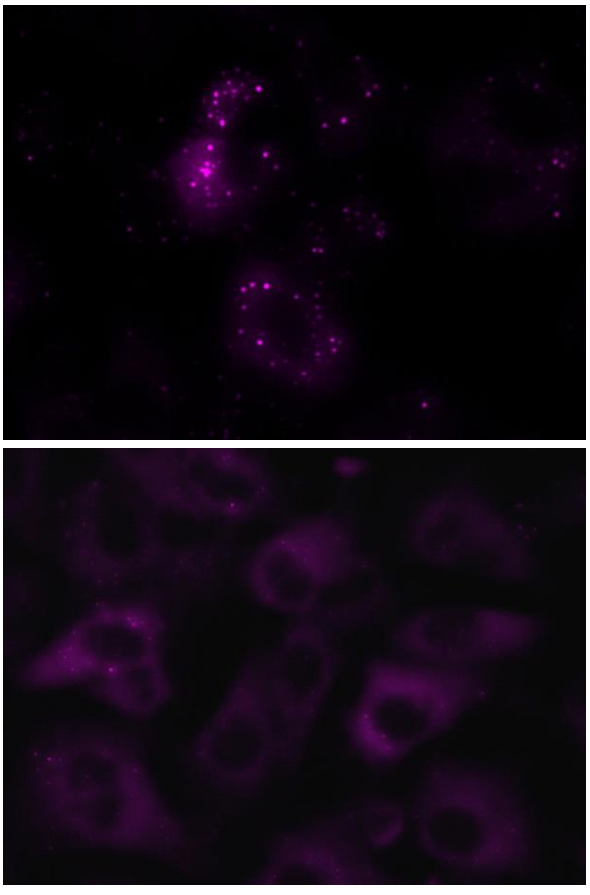
RELATED PRODUCTS

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

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde /PBS for 10 minutes at room temperature (20~25°C).
4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
5) Immerse the slide in 100 μg/mL digitonin in PBS for 10 minutes at room temperature.
6) Wash the slide in a plenty of PBS as in the step 4).
7) Add 200 μL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) onto the cells and incubate for 5 minutes at room temperature.
8) Add 200 μL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 60 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide in a plenty of PBS as in the step 4).
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; A549)

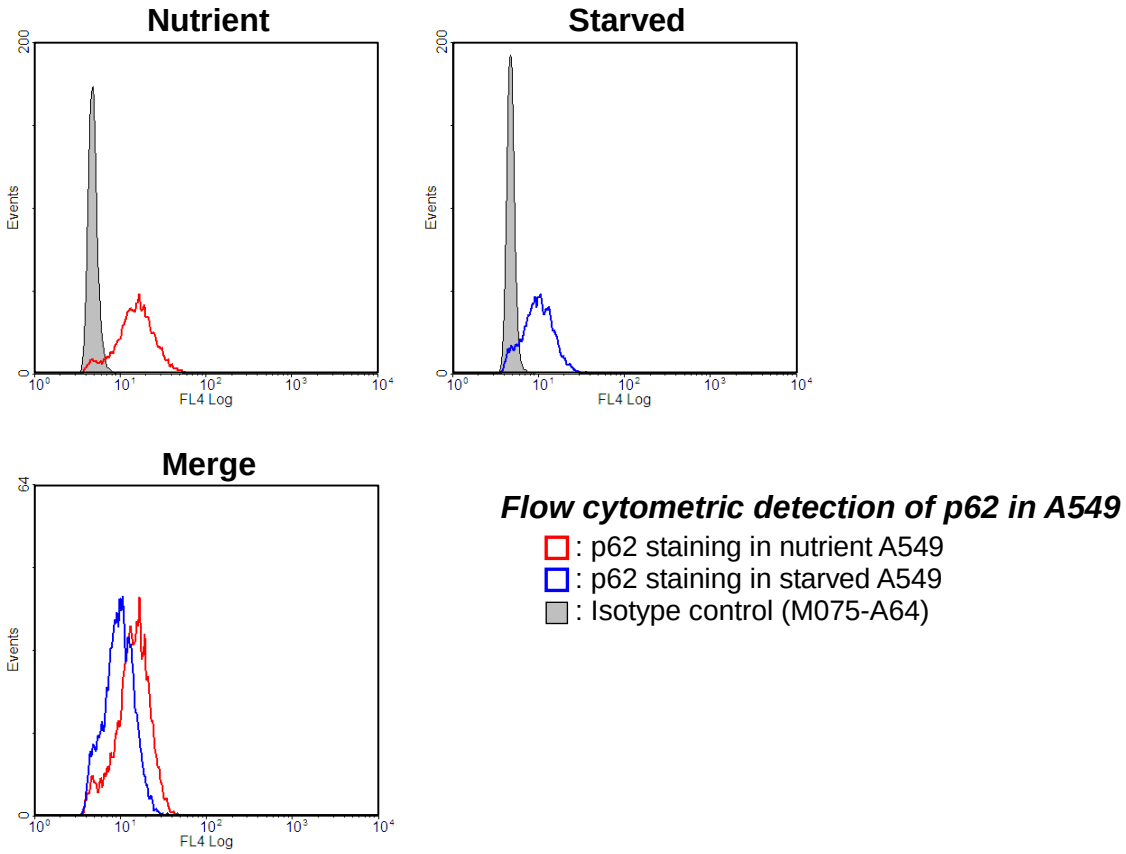


Immunocytochemical detection of p62 in A549
Upper: Starved A549
Lower: Nutrient A549

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells once with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Add 200 µL of 4% paraformaldehyde to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at room temperature.
4) Wash the cells twice with 1 mL of washing buffer.
5) Add 200 µL of 100 µg/mL digitonin in PBS to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 minutes at room temperature.
6) Wash the cells once with 1 mL of washing buffer.
7) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
8) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
9) Add 20 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
10) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer Mix well and incubate for 30 minutes at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
12) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; A549)



Flow cytometric detection of p62 in A549

: p62 staining in nutrient A549
: p62 staining in starved A549
: Isotype control (M075-A64)