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

My select sampler set

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

CODE No. M162-A64MS

CLONALITY Monoclonal
CLONE 5F2
ISOTYPE Mouse IgG1 κ
QUANTITY 20 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human p62, 120-440 aa (recombinant)
FORMURATION PBS containing 1% BSA and 0.09% NaN_3.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

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	A549	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)

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

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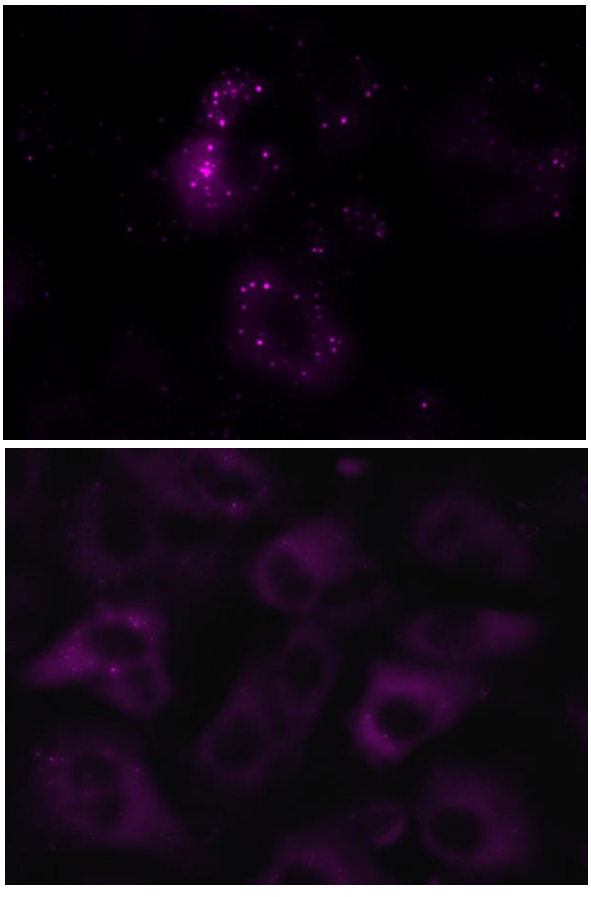
Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25^{o}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)



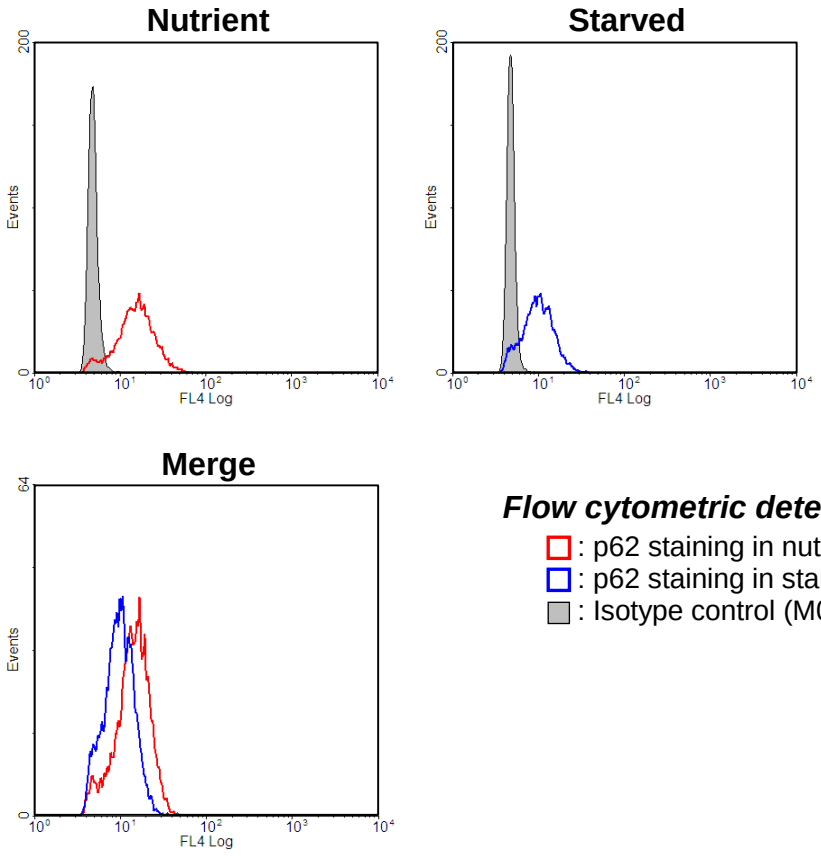
Immunocytochemical detection of p62 in A549

Upper: Starved A549
Lower: Nutrient A549

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 minutes at room temperature.
4) Wash the cells 2 times 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 1 time 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)