Technical Data Sheet

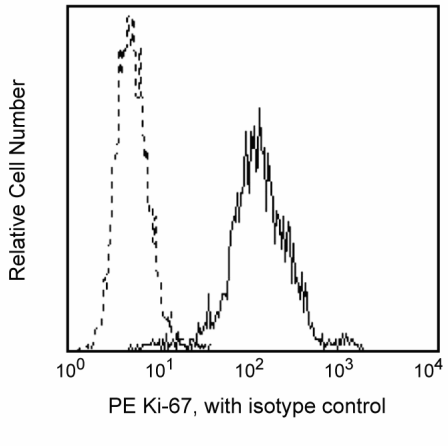
PE Mouse Anti-Human Ki-67 Set

Product Information

Material Number:	**556027**
Size:	100 tests
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Pig
Component:	**51-36525X**
Description:	PE Mouse Anti-Human Ki-67
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	B56
Immunogen:	Human Ki-67
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-35405X**
Description:	PE Mouse IgG1, κ Isotype Control
Size:	100 tests (1 ea)
Vol. per Test:	20 µl
Clone Name:	MOPC-21
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Recognizes Ki-67, a nuclear cell proliferation-associated antigen expressed in all active stages of the cell cycle. Ki-67 is revealed as a double band (345 and 395 kDa) in western blot analysis of proliferating cells. B56 was developed using an immunogen composed of the immunodominant epitope of the Ki-67 protein. Antibodies B56 and MIB 1 react with this immunogen. Flow cytometric analysis reveals that the binding of B56-PE can be blocked by MIB 1 purified antibody.



Profile of Ki-67 PE Set expressed on permeabilized MOLT-4 cell line analyzed by flow cytometry

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed by gel filtration chromatography.

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Ki-67 staining protocol by flow cytometry:

1. Harvest, count and pellet cells following standard procedures (Note: Ki-67 is expressed by the proliferative cells. You may get no staining with the resting cells, e.g. unstimulated PBMC).
2. While vortexing, add 5 ml drop by drop of cold 70% - 80% ethanol into the cells pellet (1-5 x 10e7 cells). Then incubate at -20°C for 2 hours minimum. These fixed cells can be used up to 60 days after fixing (Store at -20°C).
3. Add 30-40 ml wash buffer (PBS with 1% FBS, 0.09% NaN3 pH7.2) to the fixed cells. Centrifuge the cells for 10 minutes at 1000 rpm and aspirate supernatant. Wash one more time with 30-40 ml of wash buffer. Centrifuge at 1000 rpm for 10 minutes and aspirate supernatant.
4. Resuspend the cells to a concentration of 1 X 10e7/ ml (1 X 10e6/100 µl).
5. Transfer 100 µl cell suspension into each fresh tube.
6. Add 20 µl of properly diluted antibody according to the protocol into the tubes above. Mix gently.
7. Incubate the tubes at room temperature (RT) for 20-30 minutes in the dark.
8. Wash with 2 ml of PBS washing buffer at 1000 rpm for 5 minutes.
9. Aspirate the supernatant.
10. For direct conjugated antibody: go to steps 13 & 14.
11. For purified antibody: add 50 µl of diluted secondary antibody at optimal concentration (Cat. No. 555988), incubate at RT for 30 minutes in the dark.
12. Repeat step 8 & 9.
13. Add 0.5 ml of PBS wash buffer into each tube. For FITC conjugated antibody, add 10 µl of PI Staining Solution (Cat. No. 556463); for PE conjugated antibody, add 20 µl BD Via-Probe™ Cell Viability Solution (Cat. No. 555816) into each tube.
14. Analyze the sample with FACS.

Product Notices

1. This antibody has been optimized and preassayed with its matched isotype control to be used at the recommended volume of 20 ul/test. Titration of the reagents or substituting with other (non-matched) isotype control is NOT recommended.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Kubbutat MH, Key G, Duchrow M, Schluter C, Flad HD, Gerdes J. Epitope analysis of antibodies recognising the cell proliferation associated nuclear antigen previously defined by the antibody Ki-67 (Ki-67 protein). *J Clin Pathol.* 1994; 47(6):524-528.(Biology)

Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 1991; 39(6):741-748.(Biology)