

Technical Data Sheet

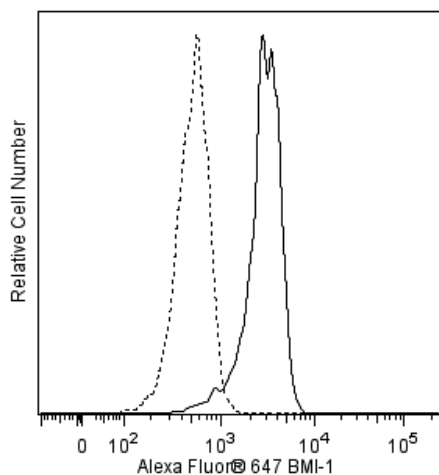
Alexa Fluor® 647 Mouse Anti-Human BMI-1

Product Information

Material Number:	562637
Alternate Name:	BMI1; PCGF4; polycomb group ring finger 4; RING finger protein 51; RNF51
Size:	50 tests
Vol. per Test:	5 µl
Clone:	P51-311
Immunogen:	Human BMI-1 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, and ≤0.09% sodium azide.

Description

The P51-311 monoclonal antibody binds to human BMI-1 (B lymphoma Mo-MLV insertion region 1 homolog). *BMI1* is a c-myc cooperating oncogene that encodes an ~45 kDa protein that is a member of the Polycomb Group (PcG) of proteins. PcG proteins are essential for the maintenance, but not initiation, of the transcriptionally repressed state of certain developmental genes. PcG proteins are a structurally diverse group of proteins with conserved functions from fly to human cells. PcG proteins form complexes and regulate the expression of genes involved in cell cycle, DNA repair and differentiation that are crucial for maintaining the self renewal of normal and cancer stem cells. Specifically, BMI-1 is a core component of PRC1 (polycomb repressive complex 1). BMI-1, via the up-regulation of hTERT and independent of c-myc, can immortalize mammary epithelial cells. BMI-1 has also been shown to repress the *INK4A* locus that controls the tumor suppressors p16 and p19ARF (mouse homologue of p14ARF) in mouse models. BMI-1 plays a role in maintaining the self-renewal capacities of stem cells including hematopoietic, intestinal, retinal and neural stem cells. During antibody development, the purified P51-311 monoclonal antibody was found to detect BMI-1 by Western blot analysis of cellular lysates and by indirect immunofluorescent staining and flow cytometric analysis of fixed and permeabilized cells.



Flow cytometric analysis of BMI-1 expression in a human osteosarcoma cell line. U-2 OS cells (ATCC; HTB-96™) were fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were stained with either Alexa Fluor® 647 Mouse IgG1, κ Isotype Control (Cat. No. 557783; dashed line histogram) or Alexa Fluor® 647 Mouse Anti-Human BMI-1 antibody (Cat. No. 562637; solid line histogram) at matched concentrations. The fluorescence histograms were derived from events with the forward and side light-scatter characteristics of intact cells. Flow cytometry was performed on a BD™ LSR II Flow Cytometry System.

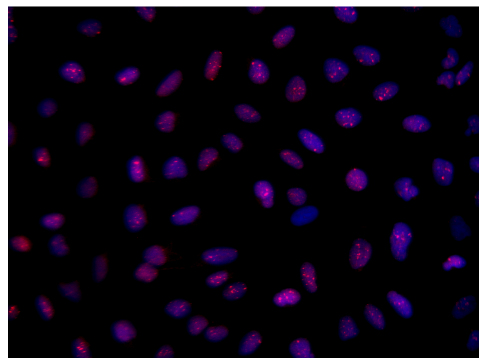


Image analysis of BMI-1 expressed in human osteosarcoma cell line. U-2 OS cells (ATCC, HTB-96™) were fixed with BD Cytotfix™ Buffer (Cat. No. 554655), permeabilized with 0.1% Triton™ X-100 (Sigma, Cat. No. X-100), and stained with Alexa Fluor® 647 Mouse Anti-Human-BMI-1 antibody (Cat. No. 562637; 5 µg/ml; pseudo-colored red). Counter-staining was with DAPI (pseudo-colored blue). The image was captured using a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ Software.

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Preparation and Storage

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

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

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Tested During Development
Immunofluorescence	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21
554655	Fixation Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
8. 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.
9. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
10. Triton is a trademark of the Dow Chemical Company.

References

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Itahana K, Zou Y, Itahana Y. Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol.* 2003; 23(1):389-401. (Biology)

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Park IK, Qian D, Kiel M, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature.* 2003; 423(6937):302-305. (Biology)

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