Technical Data Sheet

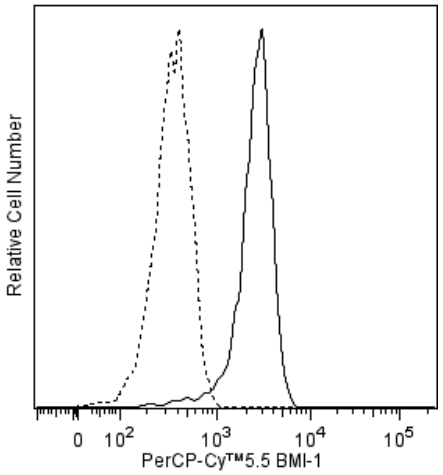
PerCP-Cy™5.5 Mouse Anti-Human BMI-1

Product Information

Material Number:	**562650**
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 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 Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050). The cells were stained with either PerCP-Cy™5.5 Mouse IgG1, κ Isotype Control (Cat. No. 550795; dashed line histogram) or PerCP-Cy™5.5 Mouse anti-Human BMI-1 antibody (Cat. No. 562650; 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 using a BD™ LSR II Flow Cytometry System.*

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 with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
554655	Fixation Buffer	100 ml	(none)

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558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 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/pharmingen/protocols for technical protocols.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
7. 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.
8. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
9. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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References

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