## **Technical Data Sheet**

# Alexa Fluor® 488 Mouse Anti-Human BMI-1

#### **Product Information**

Material Number: 562636

Alternate Name: BMI1; PCGF4; polycomb group ring finger 4; RING finger protein 51; RNF51

Immunogen: Human BMI-1 Recombinant Protein

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.

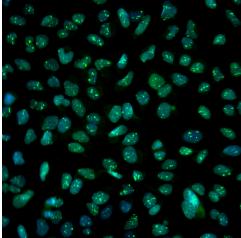
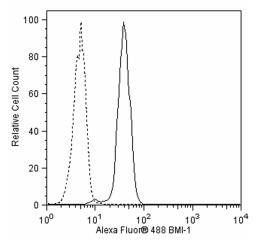


Image analysis of BMI-1 expressed in human osteosarcoma cell line. U-2 OS cells (ATCC; HTB-96™) were fixed with BD Cytofix™ Buffer (Cat. No. 554655), permeabilized with 0.1% Triton™ X-100 (Sigma, Cat. No. X-100), and stained with Alexa Fluor® 488 Mouse Anti-Human-BMI-1 antibody (5 μg/ml; pseudo-colored green). 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.



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 Phosfiow™ Perm Buffer III (Cat. No. 558050). The cells were stained with either Alexa Fluor® 488 Mouse IgG1, κ Isotype Control (dashed line histogram, Cat. No. 557782) or Alexa Fluor® 488 Mouse anti-Human BMI-1 antibody (solid line histogram, Cat. No. 562636) 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.

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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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

#### **Application Notes**

#### Application

Intracellular staining (flow cytometry)	Routinely Tested

## **Suggested Companion Products**

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

#### **Product Notices**

- This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10<sup>6</sup> 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. The Alexa Fluor®, Pacific Blue<sup>TM</sup>, 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<sup>TM</sup> dye, and Cascade Blue® dye are covered by pending and issued patents.
- 6. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
- 7. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
- 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. All other brands are trademarks of their respective owners.
- 11. Triton is a trademark of the Dow Chemical Company.

#### References

Dimri GP, Martinez JL, Jacobs JJ. The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. *Cancer Res.* 2002; 62(16):4736-4745. (Biology)

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Park IK, Qian D, Kiel M, et al. Smi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature*. 2003; 423(6937):302-305. (Biology) Sangiorgi, E., Capecchi, M. R. Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet*. 2008; 40(7):915-920. (Biology)

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562636 Rev. 1 Page 2 of 2