# Human BMI-1 Antibody

Monoclonal Mouse $IgG_{2A}$ Clone # 384515

Catalog Number: MAB33341

## DESCRIPTION

| | |
|---|---|
| **Species Reactivity** | Human |
| **Specificity** | Detects human BMI-1 in direct ELISAs and Western blots. |
| **Source** | Monoclonal Mouse $IgG_{2A}$ Clone # 384515 |
| **Purification** | Protein A or G purified from hybridoma culture supernatant |
| **Immunogen** | *E. coli*-derived recombinant human BMI-1<br>Asp96-Gly326<br>Accession # P35226 |
| **Formulation** | Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS. |

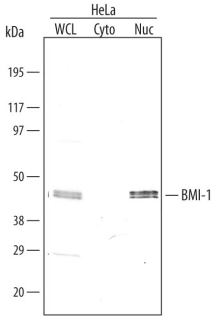
## APPLICATIONS

**Please Note:** *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

| | Recommended Concentration | Sample |
|---|---|---|
| **Western Blot** | 0.1 µg/mL | See Below |
| **Chromatin Immunoprecipitation (ChIP)** | 5 µg/5 x $10^6$ cells | See Below |
| **Intracellular Staining by Flow Cytometry** | 2.5 µg/$10^6$ cells | See Below |

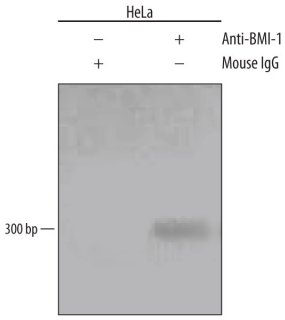
## DATA

### Western Blot



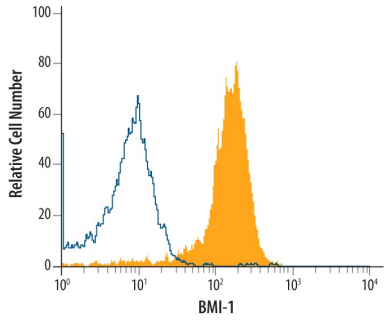

**Detection of Human BMI-1 by Western Blot.** Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line. Gels were loaded with 30 µg of whole cell lysate (WCL), 20 µg of cytoplasmic (Cyto), and 10 µg of nuclear extracts (Nuc). PVDF membrane was probed with 0.1 µg/mL Mouse Anti-Human BMI-1 Monoclonal Antibody (Catalog # MAB33341) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band for BMI-1 was detected at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

### Chromatin Immunoprecipitation (ChIP)




**Detection of BMI-1-regulated Genes by Chromatin Immunoprecipitation.** HeLa human cervical epithelial carcinoma cell line was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. BMI-1/DNA complexes were immunoprecipitated using 5 µg Mouse Anti-Human BMI-1 Monoclonal Antibody (Catalog # MAB33341) or control antibody (Catalog # MAB003) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Mouse IgG Secondary Antibody (Catalog # BAF007). Immunocomplexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *hoxc13* promoter was detected by standard PCR.

### Intracellular Staining by Flow Cytometry




**Detection of BMI-1 in HeLa Human Cell Line by Flow Cytometry.** HeLa human cervical epithelial carcinoma cell line was stained with Mouse Anti-Human BMI-1 Monoclonal Antibody (Catalog # MAB33341, filled histogram) or isotype control antibody (Catalog # MAB003, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG $F(ab')_2$ Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

## PREPARATION AND STORAGE

| | |
|---|---|
| **Reconstitution** | Reconstitute at 0.5 mg/mL in sterile PBS. |
| **Shipping** | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>● 12 months from date of receipt, -20 to -70 °C as supplied.<br>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>● 6 months, -20 to -70 °C under sterile conditions after reconstitution. |

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## BACKGROUND

BMI-1 (B cell-specific Moloney-MLV integration site #1) is a 45 kDa protooncogene that is a class II member of the Polycomb group of genes. It participates in the formation of a large multimeric complex termed PRC1 that inhibits target gene transcription. Loss of BMI-1 function precludes stem cells from self-replicating. Human BMI-1 contains an N-terminal RING-finger domain (aa 17-56), an NLS (aa 81-95) and a C-terminal Pro/Ser-rich region (aa 251-326). Human BMI-1 shares 99%, 97%, 99% and 99% aa sequence identity with bovine, mouse, feline and canine BMI-1, respectively.