

Anti-Bmi-1, clone F6

Monoclonal Antibody

Cat. # 05-637

Lot # DAM1588209

pack size: 100 µg

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Store at -20°C



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IC	H, M, R, Rb	IgG1	a.a. 1-202	M	40-44 kDa	NP_005171

Background

The Bmi-1 protein is a member of the polycomb group (PcG) proteins. PcG proteins form multiprotein complexes that function as transcriptional repressors. Several lines of evidence implicate Bmi-1 in tumorigenesis. Firstly, Bmi-1 cooperates with c-Myc in the generation of lymphomas in double transgenic mice. Secondly, Bmi-1 blocks senescence and immortalizes mouse embryo fibroblasts, although not human fibroblasts, and in combination with an activated H-ras gene leads to neoplastic transformation. Thirdly, the BMI-1 gene is amplified in certain mantle cell lymphomas and is overexpressed in a subset of non-small cell lung cancer, colorectal carcinomas, multiple myelomas and medulloblastomas, but not glioblastomas. Fourthly, the transformation of human hematopoietic progenitor cells by the oncogenic fusion protein E2a-Pbx1 requires Bmi-1. Indeed, based on a list of genes differentially expressed in a mouse model of metastatic prostate cancer on a wild-type and Bmi-1 deficient genetic background a gene signature was recently identified that may predict response to therapy and survival in multiple types of cancer.

Presentation

Purified mouse IgG1 in buffer containing 0.01M Tris-Glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide.

Concentration

1 mg/mL

Specificity

Recognizes Bmi-1 (triplet), Mr 40-44 kDa

Immunogen

Immunized with recombinant Bmi-1 protein corresponding to residues 1-202 of mouse Bmi-1.

Molecular Weight

40-44 kDa

Method of Purification

Protein G chromatography

Species Cross-reactivity

Human, mouse, rat, and rabbit

Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C.

Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

Control

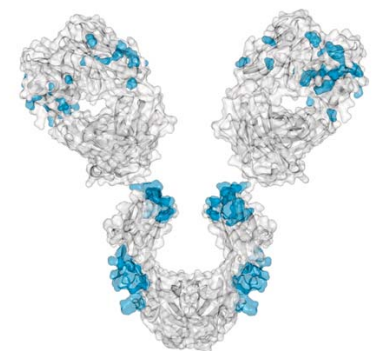
U2OS whole cell lysate, K-562 nuclear extract, 0-4 hour embryos or 12-24 hour embryos.

Quality Control Testing

Routinely evaluated by western blot on RIPA lysates from U2OS cells.

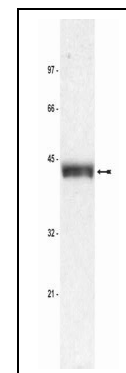
Western Blot Analysis:

0.2-2 µg/mL of this lot detected Bmi-1 in RIPA lysates from U2OS cells.



References

1. Song, Li-Bing, *et al.* (2006). *Cancer Res.* 66: 6225-32.
2. Voncken, J. W., *et al.* (1999). *J Cell Sci.* 112 (Pt 24): 4627-39.
3. Alkema, M. J., *et al.* (1997). *Genes Dev.* 11: 226-40.



Western Blot Analysis

Representative lot data. U2OS cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Bmi-1 (0.2 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates Bmi-1 (~40-44 kDa).

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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ADDITIONAL RESEARCH APPLICATIONS

Immunoprecipitation: This antibody has been reported by an independent laboratory to immunoprecipitate Bmi-1 from mouse embryo protein extracts.

Immunocytochemistry: This antibody has been reported by an independent laboratory to show positive immunostaining for Bmi-1 in U2OS cells fixed with 2% formaldehyde.

PROTOCOL**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.1% SDS, 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 1 mM NaF. Sonicate (4 pulses, each 10 sec in duration to reduce viscosity) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween®-20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.2-2 µg/mL of anti-Bmi-1, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in TBST-MLK for 0.5 hour at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBST for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

RELATED PRODUCTS (specific)

cat #	description
64-201	■ Visualizer™ Western Blot Detection Kit, mouse
12-349	■ Goat Anti-Mouse IgG, HRP conjugate
20-212	■ LumiGLO® Chemiluminescent Substrate
16-266	■ Protein G Agarose, Fast Flow
12-506	■ Goat Anti-Mouse IgG (H+L), fluorescein conjugate
12-509	■ Goat Anti-Mouse IgG (H+L), rhodamine conjugate
12-445	■ Goat Anti-Mouse IgG, biotin conjugate
17-500	■ CATCH AND RELEASE REVERSIBLE IMMUNOPRECIPITATION SYSTEM
16-266	■ PROTEIN G AGAROSE FAST FLOW 10 mL
16-125	■ PROTEIN A-AGAROSE 10 mL

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow(TM) ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ WESTERN BLOT BLOCKING REAGENT 20G

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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