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## Certificate of Analysis

### Anti-Bmi-1, clone 229F6

(mouse monoclonal IgG1)

Catalog # 05-637

Lot # 22103

**Immunogen:** Immunized with recombinant Bmi-1 protein corresponding to residues 1-324 of mouse Bmi-1.

Clone 229F6.

**Specificity:** Recognizes Bmi-1 (triplet), Mr 40-44kDa in Upstate's gel system.

**Species Cross-reactivity:** Human, mouse, rat, and rabbit.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

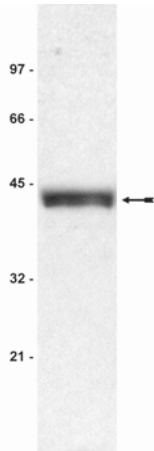
**Formulation:** 100µg of protein G purified mouse IgG in 165µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

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### Quality Control Testing

**Immunoblot Analysis:** 0.2-2µg/ml of this lot detected Bmi-1 in RIPA lysates from U2OS cells.



**Immunoblot Analysis**  
U2OS cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Bmi-1 (0.2µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Bmi-1 (40-44kDa).

### 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.

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### General References:

1. Voncken, J.W., *et. al.* *J. of Cell Science*, **112**: 4627-4639, 1999.
2. Jacobs, J.J.L., *et. al.* *Genes Dev.*, **13**: 2678-2690, 1999.
3. Jacobs, J.J.L., *et. al.* *Nature*, **397**: 164-168, 1999.
4. Alkema, M.J., *et. al.* *Genes. Dev.*, **11**: 226-240, 1997.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.1% SDS, 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM 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), with 0.1% Tween 20 (TBST-MLK) for 30 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.2-2µg/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 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).