NEUROMICS

Bmi1 Data Sheet

Catalog Number:	RA25083	**Host:**	Rabbit
Product Type:	Affinity Purified	**Species Reactivity:**	Human, Rat, Mouse, Rabbit, Chicken, Primate, Cat, Zebrafish
Immunogen Sequence:	A synthetic peptide made to an internal region (within residues 1-100) of the human Bmi1 protein. [Swiss-Prot# P35226].	**Format:**	Liquid. Tris-glycine, 150mM NaCl and 0.05% Sodium Azide as a preservative. Concentration: 1.09 mg/ml.
Applications:	Immunocytochemistry 1:50-1:200 Immunofluorescence: 1:50-1:200 Western blot: 1:50-1:200 *Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		
Publications:	Mori, T., et al, Mol. Cell Biol., 25(12): 5183-5195 (2005)2. Satijn, DPE., et al, Mol. Cell Biol., 17(7): 4105-4113 (1997)3. Obuse, C., et al, Genes to Cells, 9: 105-120 (2004		

Application Notes

This antibody can be used for Western blot where a band is seen ~37 kDa. There are also some less intense non-specific bands that do not interfere with the Bmi1 band. This antibody is also useful for immunocytochemistry/immunofluorescence.

Western Blot:

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Bmi1 primary antibody (NB 110-40823) in blocking buffer and incubate 1.5 hours at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce's#39;s ECL).

FOR RESEARCH USE ONLY

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry:
Culture cells to appropriate density in 35mm culture dishes or 6-well plates.

1. Pull off culture medium with and add 10% formalin to the dish. Fix at room temperature for 30 minutes..
2. Take off the formalin and add ice cold methanol (kept in well sealed bottle in -20C). Incubate for 5-10 minutes.
3. Take off methanol and add PBS (You can add 0.1% Tween-20 to PBS used here and all subsequent steps), be sure to not let the specimen dry out. Wash 3 times 10 minutes before proceeding to blocking step.
4. To block nonspecific antibody binding incubate in 10% normal goat serum for a minimum of 1 hr at room temp. Cells can also block overnight at 4oC for this step.
5. Add primary antibody at appropriate dilution and incubate at room temp for 2 hrs or overnight at room temp.
6. Remove primary antibody and replace with PBS. Wash 3 x 10 min in PBS.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hr at room temperature
8. Remove antibody and replace with PBS, wash 1 x 10 min in PBS. Add Hoechst 33258 to PBS at 1:25,0000 and incubate for 10 min. Wash a third time with PBS for 10 min (total of 3X10min PBS washes).
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide and parafilmed. Cells can also be coverslipped using Fluoromount. If storing coverslip be sure to seal the edges with clear nail polish.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Image: Bmi1 staining in Hela cells.

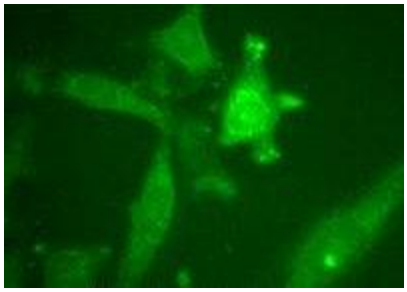
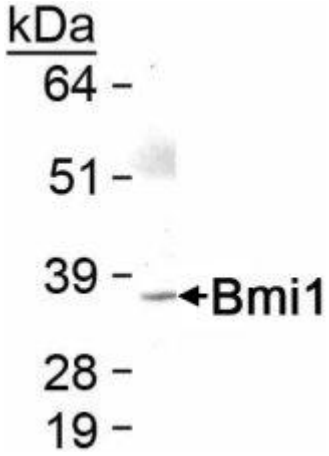


Image: Detection of Bmi1 protein in skeletal muscle.



FOR RESEARCH USE ONLY

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com