# Product Datasheet

## BMI-1 Antibody (LLBmi1-1) - BSA Free
## NBP1-96140

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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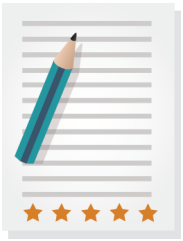
Updated 10/23/2024 v.20.1





# NBP1-96140

BMI-1 Antibody (LLBmi1-1) - BSA Free

## Product Information

| | |
|---|---|
| **Unit Size** | 0.1 ml |
| **Concentration** | 1.0 mg/ml |
| **Storage** | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles. |
| **Clonality** | Monoclonal |
| **Clone** | LLBmi1-1 |
| **Preservative** | 0.05% Sodium Azide |
| **Isotype** | IgG2b Kappa |
| **Purity** | Protein G purified |
| **Buffer** | PBS |
| **Target Molecular Weight** | 42 kDa |

## Product Description

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|---|---|
| **Host** | Mouse |
| **Gene ID** | 648 |
| **Gene Symbol** | BMI1 |
| **Species** | Human, Mouse |
| **Immunogen** | Human Bmi1 protein [Swiss-Prot# P35226] |

## Product Application Details

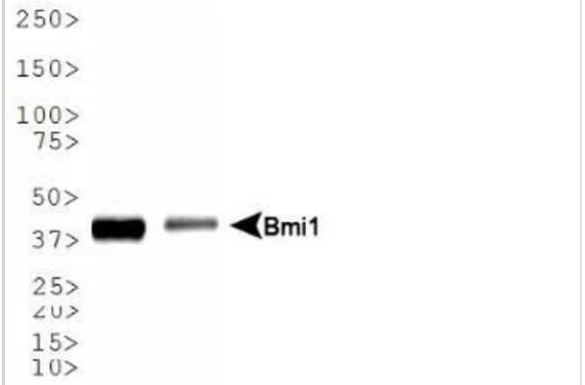
| | |
|---|---|
| **Applications** | Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP) |
| **Recommended Dilutions** | Western Blot 1:1000, Flow Cytometry, Immunocytochemistry/ Immunofluorescence 1:100, Immunoprecipitation 1:10-1:500, Flow (Intracellular), Chromatin Immunoprecipitation (ChIP) 1:10-1:500 |
| **Application Notes** | This Bmi1 Antibody (LLBmi1-1) is useful for Immunocytochemistry/Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation and Western blot, where a band can be seen at approximately 42 kDa. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. |

## Images

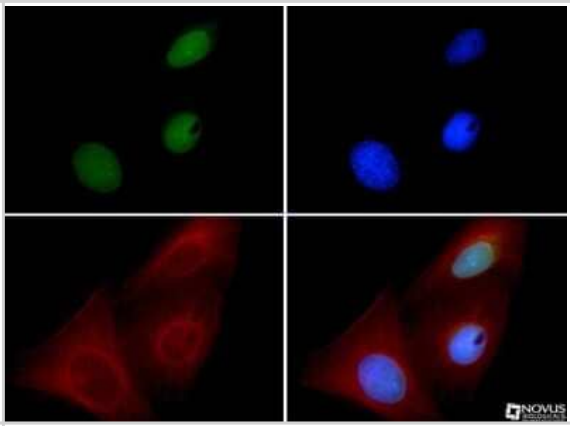
Immunocytochemistry/Immunofluorescence: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NBP1-96140 at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

Immunocytochemistry/Immunofluorescence: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - NIH3T3 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NBP1-96140 at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.

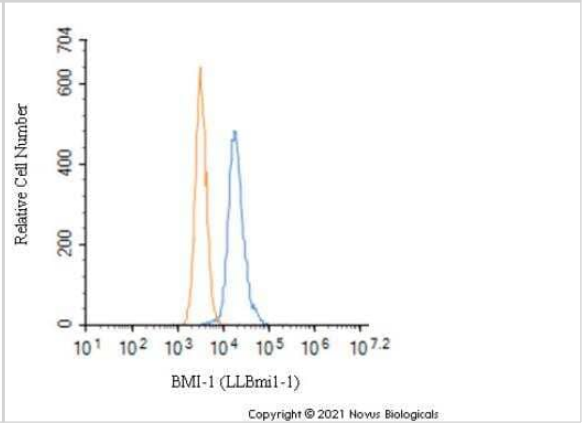

Western Blot: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - Western blot analysis of Bmi1 expression in 1) U2OS and 2) K562 whole cell lysates using NBP1-96140.

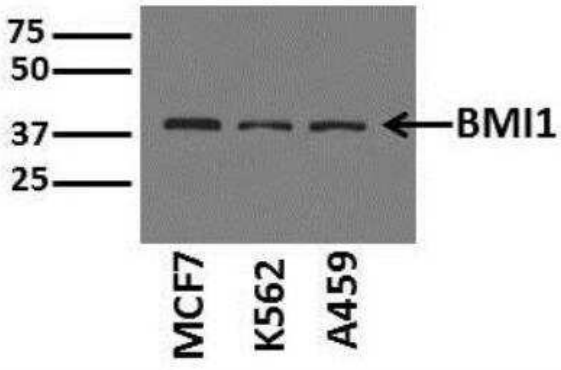
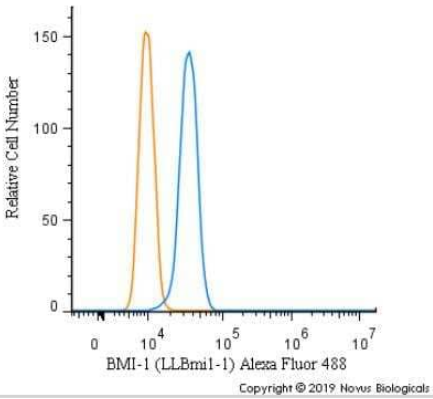
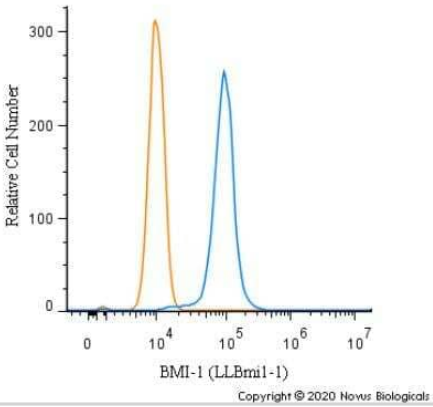
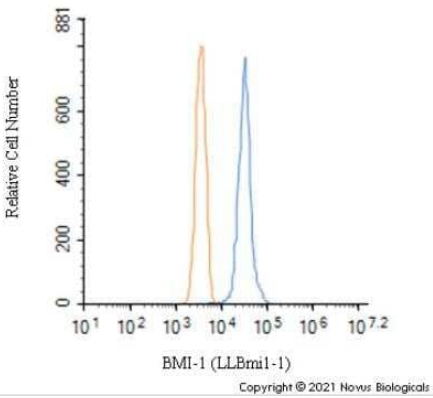



Immunocytochemistry/Immunofluorescence: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - Bmi1 antibody was tested in U2OS cells with FITC (green). Nuclei and alpha-tubulin were counterstained with Dapi (blue) and Dylight 550 (red).



Flow Cytometry: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - An intracellular stain was performed on NIH3T3 cells with BMI-1 Antibody (LLBmi1-1) NBP1-96140 (blue) and a matched mouse IgG2b Kappa isotype control (orange) MAB004. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).



| | |
|---|---|
| Western Blot: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - BMI-1 expression in human cell lines (MCF-7, K562 and A459). Image from verified customer review. |  |
| Flow Cytometry: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - An intracellular stain was performed on U2OS cells with BMI-1 Antibody [LLBmi1-1] NBP1-96140AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488. |  |
| Flow Cytometry: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - An intracellular stain was performed on U2OS cells with BMI-1 [LLBmi1-1] Antibody NBP1-96140 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher). |  |
| Flow Cytometry: BMI-1 Antibody (LLBmi1-1) [NBP1-96140] - An intracellular stain was performed on U937 cells with BMI-1 Antibody (LLBmi1-1) NBP1-96140 (blue) and a matched mouse IgG2b Kappa isotype control (orange) MAB004. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher). |  |

## Publications

Chen SM, Wang BY, Lee CH et al. Hinokitiol up-regulates miR-494-3p to suppress BMI1 expression and inhibits self-renewal of breast cancer stem/progenitor cells Oncotarget 2017-09-29 [PMID: 29100291] (WB, Human)

Courel M, Friesenhahn L, Lees JA. E2f6 and Bmi1 cooperate in axial skeletal development. Dev Dyn. 237(5):1232-42. 2008-05-01 [PMID: 18366140] (Chemotaxis, Mouse)

## Procedures

**Protocol Specific for Bmi1 Antibody (LLBmi1-1) [NBP1-96140]**
BMI-1 Antibody (LLBmi1-1):
Western Blot Protocol
1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 three 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.
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.

6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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

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## Products Related to NBP1-96140

| | |
|---|---|
| HAF007 | Goat anti-Mouse IgG Secondary Antibody [HRP] |
| NB720-B | Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin] |
| NBP1-43317-0.5mg | Mouse IgG2b Kappa Light Chain Isotype Control (MG2b) |
| NBP1-96140AF488 | BMI-1 Antibody (LLBmi1-1) [Alexa Fluor® 488] |

### Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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