

Detection of Bmi-1 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Serum: Normal Rat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 012-000-001

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Purified Mouse Anti-Bmi1 Monoclonal Antibody

Millipore

Billerica, Massachusetts 01821

www.millipore.com

1-800-645-5476

Catalog # 05-637

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdpharma.com

1-877-232-8995

Catalog # 557273

Secondary Antibody: Biotin Rat Anti-Mouse IgG1

BD Biosciences

San Jose, CA 95131

www.bdpharma.com

1-877-232-8995

Catalog # 550331

Label Complex: Vectastain Elite ABC Kit (Standard)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6100

Staining Procedure

Positive Control Tissue: Liver – bile ducts

Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer (Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5

Cool for 1 minute. (Add more citrate buffer, if necessary.)

Microwave for 5 minutes at power level 5. *Temp after Microwaving* _____

Cool 20 minutes at room temperature.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse slides in 2 changes of 1X Wash Buffer for 5 minutes each.

6. Block with 10% Normal Rat Serum for 20 minutes at room temperature.

Lot # _____ Date Reconstituted _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at 1:50 dilution, and incubate overnight at 4°C.
Lot # _____ Date Aliquoted _____

For negative control slides, dilute the protein concentration of the mouse IgG1 serum to match that of the primary antibody, if necessary. Make a 1:50 dilution from this normalized serum, and apply to the slides. Incubate overnight at 4°C.

Lot # _____ Date Reconstituted _____

*****Next Day*****

9. Bring the slides up to room temperature in 1X Wash Buffer for at least 15 minutes.
10. Apply the rat anti-mouse secondary antibody at a 1:1000 dilution, and incubate for 30 minutes at room temperature.
Lot # _____ Date Reconstituted _____
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the label complex from the Standard Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
Exp. Date _____ New Kit: yes / no
13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.
14. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp. Date _____ New Kit: yes / no
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with Harris Hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X Wash Buffer until tissues turn blue.
19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip