

# Detection of p21 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## **Reagent and Antibody Information**

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

### Blocking Serum: Normal Horse Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 008-000-001

### Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

### Primary Antibody: Mouse Monoclonal p21 Antibody (F-5)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

[www.scbt.com](http://www.scbt.com)

1-800-457-3801

Catalog # sc-6246

### Negative Control Serum: Purified Mouse IgG2b Isotype Control Serum

BD Biosciences

San Jose, CA 95131

[www.bdbiosciences.com](http://www.bdbiosciences.com)

1-855-236-2772

Catalog # 557351

### Secondary Antibody: Biotinylated Horse Anti-Mouse IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # BA-2001

Label Complex: R.T.U. Vectastain Elite ABC Reagent  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # PK-7100

### **Staining Procedure**

Positive Control Tissue: Pancreas, liver, and lung  
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 slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

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)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_

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

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

6. Block with 10% normal horse 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 the primary antibody at a 1:10 dilution. Incubate overnight at 4°C.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute mouse IgG2b control serum so that it's IgG2b protein concentration matches that of the primary antibody (if necessary). Then make a 1:10 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate overnight at 4°C.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

\*\*\*\*\*Next Day\*\*\*\*\*

9. Bring the slides up to room temperature in 1X wash buffer for at least 15 minutes.

10. Apply the horse anti-mouse secondary antibody at a 1:500 dilution. 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 Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

Exp. Date \_\_\_\_\_ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen. 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 hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X wash buffer until the 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

Updated 10/20/04