Detection of p19 ARF in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
Normal Goat IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Staining Kit: Goat ABC Staining System
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-2023

Note: This kit contains all reagents necessary to make the blocking reagent and label complex.

Avidin / Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary Antibody: Goat Polyclonal p19 ARF Antibody (G-19)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-7403

Staining Procedure

Positive Control Tissue: 7-day post-natal 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 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 again for 5 minutes at power level 5. *Temperature Before Cooling Slides*_____
   Cool 20 minutes at room temperature.
   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.

   Goat ABC Staining Kit
   Lot #_____ Exp. Date (1 year from received date) _____

6. Apply the blocking reagent from the Goat Staining Kit for 1 hour at room temperature.
   (Made with 75 µl donkey serum (blue cap) and 5 ml diluent)

   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 SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

8. Apply the primary antibody at a 1:150 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date_____

For negative control slides, dilute normal goat IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:150 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 for 1 hour at room temperature.
Lot #_____ Exp. Date _____

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

10. Apply the secondary antibody from the Goat Staining Kit. Incubate for 30 minutes at room temperature. (Made with 75 µl donkey serum, 25 µl of donkey anti-goat IgG (green cap), and 5 ml diluent)

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

12. Apply the label complex from the Goat Staining Kit. Incubate for 30 minutes at room temperature. (Made with 50 µl reagent A (white cap), 50 µl reagent B (purple cap), and 2.5 ml diluent)

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

14. Apply the DAB chromogen 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 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 08/03/04