

Detection of Ini1 in Formalin-Fixed, Paraffin-Embedded Human Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Serum: Normal Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 017-000-011

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rabbit Polyclonal Ini1 Antibody (H-116)

Santa Cruz Biotechnology

Santa Cruz, CA 95060

www.scbt.com

1-800-457-3801

Catalog # sc-13055

Secondary Antibody: Donkey Anti-Rabbit IgG (H+L) Biotin-SP-Conjugated

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 711-065-152

Label Complex: Peroxidase-Conjugated Streptavidin SS Label

Biogenex Laboratories

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Teratoma

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 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 donkey 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 a 1:25 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp Date _____

For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:25 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 donkey anti-rabbit secondary antibody at a 1:1000 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 Streptavidin SS Label. Incubate for 30 minutes at room temperature.

Lot # _____ Exp Date _____

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 03/07/07