

Detection of KAI-1 in Frozen Human Prostate Tissue

Antibody Information:

Normal Horse Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #008-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Normal Mouse Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #015-000-001

Primary antibody: (C33, mouse anti-KAI-1)

Provided by Barrett lab at NIEHS

Suggested dilution: 1:100

Secondary antibody: Biotinylated horse anti-mouse IgG

Vector Mouse Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6102

Label antibody

Vector Elite Mouse Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6102

Equipment:
Black and Decker
Flavor Scenter Steamer Plus

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Staining Procedure

- Positive Control Tissue: Liver, Intestine
- Stain localization: Cell Membrane

For Frozen Tissue Sections

Two sequential 6 micron sections were cut per slide (Probe-On Plus by Diagger). Sections are cut and immediately fixed in Rapid Fix (Shandon-Lipshaw) for 7 seconds. Place section in 1X AB. After the last section is cut, wash in 1X AB for 5 minutes. Repeat buffer wash.

1. Quench endogenous peroxidase by placing slides in 0.3% hydrogen peroxide for 30 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Perform Heat Induced Epitope Retrieval using Steamer
Place a full rack of slides in Tissue Tek container containing 200 mls 1X citrate buffer.
Steam samples for 30 minutes Temp. _____
Cool 20 minutes at room temperature
Rinse in distilled water 3 changes for 2 minutes each
Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
4. Block using 5% Normal Horse Serum for 20 minutes.
Lot# _____ Reconstituted Date _____
Wipe excess reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.
5. Apply Avidin/Biotin block Lot# _____ exp _____ New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody
6. Apply primary antibody (C33, mouse anti-KAI-1) at a 1:100 dilution for 1 hour.
Lot# _____ exp _____

On negative control slides, apply Normal Mouse Serum at a 1:100 dilution for 1 hour.

NOTE: Add 2% Normal Horse Serum final concentration to this dilution.

Lot# _____ reconstituted _____

Lot# _____ reconstituted _____

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

8. Apply the secondary antibody from kit and incubate for 30 minutes. (prepare at least 30 mins prior to use)

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

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

10. Apply the label antibody from kit and incubate for 30 minutes.

11. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)

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

12. Rinse in tap water 3 minutes.

13. Counterstain with Modified Harris Hematoxylin for 30 seconds.

14. Rinse in tap water until water is clear.

15. Place slides in 1X Automation buffer for one minute with gentle agitation to blue slides.

16. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

17. Coverslip using Permount^o

updated 8/8/2003
