

Detection of GSTpi in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

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

Primary antibody: Rabbit anti-GSTpi
Novocastra (Distributed by Vector Laboratories)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #NCL-GSTpi

Negative: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

Secondary: Vector Rabbit Elite ABC

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6101

Label antibody: Vector Rabbit Elite ABC

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6101

Staining Procedure

- Positive Control Tissue: Rat liver tissue containing foci
- Stain localization: Cytoplasmic and Nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Perform Heat Induced Epitope Retrieval using Microwave Oven.
Place a full rack of slides in a container containing 200 mls distilled water.
MWO for 5 minutes at level 5
Cool for 1 minute (Add 50 mls distilled water to container if needed)
MWO for 5 minutes at level 5 Temp _____
Cool 20 minutes at room temperature.
Rinse in distilled water 3 X 2 minutes each
Place slides in buffer for 5 minutes
4. Block using 5% normal goat serum for 20 minutes.
Lot# _____ Reconstituted Date _____

DO NOT RINSE SLIDES.

5. Apply Avidin/Biotin block

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

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody GSTpi and incubate according to tissue type.

Lot# _____ Aliquoted yes / no Date Aliquoted _____

For LIVER tissue: Suggested dilution 1:800 Incubation time: 1 hour at room temp.

For LUNG tissue: Suggested dilution 1:500 Incubation time : 1 hour at room temp.

For the negative control slides, match the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (GSTpi) and use this to make the suggested dilution. Apply to the slides and incubate for one hour.

Lot # _____ Reconstituted Date _____

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

8. Apply secondary antibody from Vector Rabbit Elite Kit and incubate for 30 minutes.

Exp. Date _____ New Kit: yes / no

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

10. Apply Label antibody from Vector Rabbit Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)

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

12. 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

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

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

17. Dehydrate through the following solutions.

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

18. Coverslip

updated 10/12/05