

# Detection of SDHB in Formalin-Fixed, Paraffin-Embedded Mouse 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](#)

Staining Kit: ImmPRESS Anti-Rabbit Ig (peroxidase) Polymer Detection Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog # MP-7401

**Note:** This kit contains a pre-dilute blocking serum and polymer reagent.

Primary Antibody: Rabbit Polyclonal Anti-SDHB Antibody (FL-280)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog # sc-25851

## Staining Procedure

Positive Control Tissue: Liver  
Stain Localization: Cytoplasmic

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 Steamer  
Add distilled water to the bottom portion of the steamer.  
Preheat 200ml of 1X citrate buffer in a Tissue Tek® container in the steamer between 95°C and 100°C.  
Immerse a full rack of slides into the citrate buffer and place the lid back on the steamer.  
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)  
Steam the slides for 20 minutes.  
Remove container from steamer and cool for 20 minutes.  
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.
6. Block with the 2.5% normal horse serum from the ImmPRESS kit for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:100 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 1:100 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 \_\_\_\_\_

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Apply the anti-rabbit polymer from the ImmPRESS kit, and incubate for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
11. 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
12. Rinse the slides in tap water 3 minutes.
13. Counterstain with hematoxylin for 20 seconds.
14. Rinse the slides in tap water until water is clear.
15. Gently agitate slides in 1X wash buffer until the tissues turn blue.
16. Dehydrate through the following solutions:

<b>Solutions</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

17. Coverslip

*Updated 07/24/13*