

Detection of Brachyury 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: ImmPRESS Anti-Goat Ig (peroxidase) Polymer Detection Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # MP-7405

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

Primary Antibody: Goat Anti-Brachyury Polyclonal Antibody (N-19)

Santa Cruz Biotechnology

Santa Cruz, CA 95060

www.scbt.com

1-800-457-3801

Catalog # sc-17743

Staining Procedure

Positive Control Tissue: Embryo – 8.5
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. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)
Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* _____
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.
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 goat 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-goat polymer from the ImmPRESS kit, and incubate for 30 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:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

17. Coverslip

Updated 11/25/13