

Microwave Immunohistochemistry On Mohs Cryostat Sections

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With the advent of Mohs surgery in the dermatology clinics, making sure the margins are clear of any cancer is of the utmost importance to both patient and dermatopathologist. While the patient is still in the surgery suite, immunohistochemistry staining can be done in the laboratory microwave (Pelco microwave with waiter load cooler wattage controller and temperature probe). The immunohistochemistry procedure takes only 25-30 minutes as compared to the more conventional methods that take 2 hours or more.

The microwave needs to be checked for hot spots using the Pelco #36140 microwave bulb array. Make sure the area in which you are going to put your slides has no hot spots, indicated by no illuminating bulbs. Using a Sigma Diagnostics #H6644 Humid Chamber; place a paper towel in the bottom of the chamber and fill with ¼ inch of water. This is in addition to the water loads used to eliminate hot spots. Cut a very small hole into the top of the humid chamber—just large enough for the probe to fit into—at the exact point that the probe will be immersed in the reagent that is covering the tissue section. The temperature probe will measure the reagent temperature on tile slide, maintaining a constant temperature of 40°C. You can run up to six slides at once in a single humid chamber.

Vaporization is very important to this procedure. The vapor is contained in the humid chamber and enhances the reaction. Reagents used are tile Vector Universal Elite Kit, Vector

DAB kit and the DAKO HMB45 primary antibody for Melanoma.

Microwave Procedure for Immunohistochemistry

1. Cut frozen section. Air-dry for 1 minute. Fix in microwave for 3 seconds (place slide on top of weigh boat containing crushed ice, microwave on 100%).
2. Fix for 30 seconds on 95% alcohol.
3. Put slides in PBS (in coplin jar) for 1 minute.
4. Put slides in humid chamber. Apply normal serum. Set microwave at 40°C at 250 watts for 2 minutes. Make sure that temperature probe is in the reagent on the slide. Then set for another 2 minutes in the hold mode. (This setting does not emit microwaves).
5. Blot off excess reagent.
6. Apply primary antibody at 40°C at 350 watts for 2 minutes. Set for another 2 minutes in the hold mode.
7. Rinse in PBS for 1 minute and change to new PBS and rinse for another minute.
8. Apply secondary antibody, 2 minutes at 40°C at 350 watts. Set for another 2 minutes in hold mode.
9. Rinse as listed in step (7).
10. ABC reagent at 40°C at 350 watts for 2 minutes. Set for another 2 minutes in hold mode.
11. Rinse as listed in step (7).
12. DAB reagent, develop to stainer's satisfaction. This can be done in the microwave at 40°C at 350 watts, starting at 10-30 seconds (or at RT).
13. Counter stain in dilute hematoxylin, if desired, for 30 seconds.
14. Dehydrate, clear and mount. ■

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