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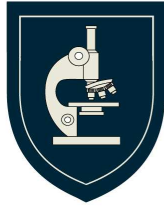
Mart-1 Staining Protocol

Reagent	Time
Acetone	2 Minutes
Tris Wash	30 Seconds
Mart-1 Antibody	11 Minutes
Tris Wash	20 Seconds
Poly-HRP	10 Minutes
Tris Wash	20 Seconds
DAB	2 Minutes
Distilled Water	Quick Rinse
Hematoxylin Gill 3	2 Sec Dip
Water	Rinse
Bluing	2 Sec Dip
Water	Rinse
100% Alcohol	4 Stainer Wells

Ordering Information

Reagent	Company	Product #
Mart-1 Primary Antibody 1 ml	Lab Vision 1-800-828-1628	MS-716-P ~ \$500
Polymer Based IHC Detection System Poly HR, DAB, Blocking	Vision Biosystems 1-800-248-0123	PV6113 ~ \$1,000
Tris-Buffered Saline (TBS) 6 packs = 6 liters	Dako 1-800-235-5763	S300130-2 ~ \$120





Mart-1 Staining Protocol for Frozen Sections

Preparing the Slides

- Cut the sections 4-5 microns and use charged slides
- Have an H&E and Mart-1 slide for each section of tissue
 - H&E - The first sections cut to ensure all the skin edge is present
 - Mart-1 - At least 4 sections on the slide
- Place the tissue near the top of the slide because the bottom often gets over rinsed from dipping in the Tris
- Control Slide
 - There must be a control slide each time the Mart-1 protocol is used
 - If available, use a section of known melanoma as the positive control
 - Normal skin can be used to check for positive reactivity of the melanocytes
 - For the negative control slide, skip the Mart-1 antibody step
- Apply the liquid blocker pen closely around the tissue
- Heat the slides for at least 5 minutes
- Cool at room temperature for at least 5 minutes

Staining Protocol

1. **Acetone:** 2 minutes
2. **Tris Wash:** 30 seconds
 - Add one pack of the Tris-buffered saline to one liter of distilled water
 - Store in a plastic container labeled as Tris. The six packs will last about 90 cases
 - Move all the slides into the Tris Rinse Coplin jar
 - Always shake off the reagents before placing the slides in the Tris Rinse
 - Slowly dip each slide for 30 seconds in the Tris Coplin jar
3. **Mart-1:** 11 minutes
 - Dilution: 1 ml Mart-1 Antibody + 50 ml blocking solution
 - Store in a glass bottle. The Mart-1 solution will last about 30 cases
 - Slowly mix the Mart-1 with a pipette
 - Be conservative and apply just enough to completely cover the tissue
4. **Tris Wash:** 20 seconds
 - Move all the slides into the Tris Rinse
 - Slowly dip each slide for 20 seconds in the Tris
5. **Poly-HRP:** 10 minutes
 - The IHC detection kit will last about 70 cases
 - Apply the HRP to the slides
6. **Tris Wash:** 20 seconds
 - Move all the slides into the Tris Rinse
 - Slowly dip the slides in the Tris for 20 seconds
7. **DAB:** 2 minutes
 - 2 drops DAB Substrate + 2 drops DAB chromogen + 1 ml distiller water
 - Combine in a small plastic vial
 - Quickly mix the solution with a pipette
 - Apply the entire mixture to the slides
8. **Distilled Water Rinse**
 - Promptly add all the slides into a cup of distilled water
9. **Counterstain and Coverslip**
 - Two slow dips in the Hematoxylin Gill 3, and completely rinse off
 - Two slow dips in the bluing, and completely rinse off
 - Clip the slides onto the automated stainer and run in four alcohol wells
 - Rinse with a clearing agent and coverslip