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Part 3590
Revised January 2020

Mismatch Repair (MMR) Positive Control Slides – Technical Memo

CONTROL SLIDES: **Part 3590B**
98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining normal colon.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: MLH1, MSH2, MSH6, PMS2 quality control stained slides included.
Reactivity: Guaranteed product specific reactivity for six months from date of receipt. Revalidate after six months to verify continued reactivity.
Storage: 15-30°C in a light deprived and humidity controlled environment.
Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

APPLICATION:

Newcomer Supply Mismatch Repair (MMR) Positive Control Slides provide a single tissue source that expresses positive reactivity in each of the MMR panel of four markers; MLH1, MSH2, MSH6 and PMS2. Mismatch Repair testing is useful in screening for colorectal carcinoma (CRC), Microsatellite Instability (MSI) and Lynch Syndrome (LS).

NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. Heat dry sections in oven according to your laboratory protocol.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
    a. *See Procedure Note #1.*
3. Proceed, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
4. Rinse in distilled water; tap off excess water.
5. Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
6. Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
    a. *See Procedure Note #2.*
7. Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
    a. *See Procedure Note #3.*
8. Tap off excess buffer; apply MLH1, MSH2, MSH6 and PMS2 primary antibodies. Apply each antibody to an individual slide and tissue section. Incubate each at room temperature for 30 minutes.
9. Rinse slides in two changes of buffer.
10. Tap off excess buffer; apply HRP-Polymer solution. Incubate for 20 minutes.
11. Rinse slides in two changes of buffer.
12. Prepare required quantity of DAB substrate/chromogen.
13. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
14. Rinse slides in two changes of buffer.
15. Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
16. Rinse slides in warm tap water to blue sections.
17. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

MLH1 positive expression	Brown nuclear staining
MSH2 positive expression	Brown nuclear staining
MSH6 positive expression	Brown nuclear staining
PMS2 positive expression	Brown nuclear staining

PROCEDURE NOTES:

1. Do not allow sections to dry out at any point during procedure.
2. Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with distilled water to a 3% (1/10) solution prior to use.
3. Dilute sufficient Tris Buffered Saline 0.05M, pH 7.6, 10X (Part 140304) with distilled water to a 1/10 solution prior to use for all buffer rinses in this procedure.
4. Biocare MLH1 (G168-15) is the concentrated primary antibody used. Dilute primary antibody to 1/100 working dilution with Biocare Van Gogh Yellow Diluent (PD902).
5. Biocare MSH2 (FE11) is the concentrated primary antibody used. Dilute primary antibody to 1/400 working dilution with Biocare Renoir Red Diluent (PD904).
6. Biocare MSH6 (BC/44) is the concentrated primary antibody used. Dilute primary antibody to 1/250 working dilution with Biocare Van Gogh Yellow Diluent.
7. Biocare PMS2 (A16-4) is the concentrated primary antibody used. Dilute primary antibody to 1/200 working dilution with Biocare Renoir Red Diluent.
8. Biocare Mach 2™ Universal HRP-Polymer Detection (M2U522) is the HRP-Polymer solution used.
9. Cell Marque DAB Substrate Kit (957D) is the chromogen used.
10. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Biocare MLH1, MSH2, MSH6 and PMS2 Antibody datasheets.
2. Biocare Van Gogh Yellow Diluent datasheet.
3. Biocare Renoir Red Diluent datasheet.
4. Biocare Mach 2™ Universal HRP-Polymer Detection datasheet.
5. Cell Marque DAB Substrate Kit datasheet.
6. Modifications developed by Newcomer Supply Laboratory.