

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:

- Heat dry sections in oven according to your laboratory protocol.
- 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.
 - See Procedure Note #1.
- Proceed, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
- Rinse in distilled water; tap off excess water.
- Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
- Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
 - See Procedure Note #2.
- Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
 - See Procedure Note #3.
- 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.
- Rinse slides in two changes of buffer.
- Tap off excess buffer; apply HRP-Polymer solution. Incubate for 20 minutes.
- Rinse slides in two changes of buffer.
- Prepare required quantity of DAB substrate/chromogen.
- Tap off excess buffer; apply DAB. Incubate for 5 minutes.
- Rinse slides in two changes of buffer.
- Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
- Rinse slides in warm tap water to blue sections.
- 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:

- Do not allow sections to dry out at any point during procedure.
- Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with distilled water to a 3% (1/10) solution prior to use.
- 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.
- 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).
- Biocare MSH2 (FE11) is the concentrated primary antibody used. Dilute primary antibody to 1/400 working dilution with Biocare Renoir Red Diluent (PD904).
- Biocare MSH6 (BC/44) is the concentrated primary antibody used. Dilute primary antibody to 1/250 working dilution with Biocare Van Gogh Yellow Diluent.
- Biocare PMS2 (A16-4) is the concentrated primary antibody used. Dilute primary antibody to 1/200 working dilution with Biocare Renoir Red Diluent.
- Biocare Mach 2™ Universal HRP-Polymer Detection (M2U522) is the HRP-Polymer solution used.
- Cell Marque DAB Substrate Kit (957D) is the chromogen used.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

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

