

Mismatch Repair (MMR) Positive Control Slides – Technical Memo

CONTROL SLIDES: Part 3590B
98 Slide/Set

Mismatch Repair (MMR) Positive Control Slides contain a section of positive staining normal colon.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first and last slides within the control box contain sections of the positive control tissue stained with the Mismatch Repair (MMR) panel of four markers (MLH1, MSH2, MSH6 and PMS2) and provided for your reference. **Before using the unstained slides, review the enclosed stained slides with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for six months from the date of receipt. Revalidate after six months to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

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).

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

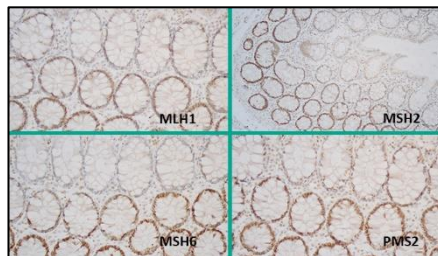
Technique: Paraffin sections cut at 4 microns on Superfrost[®] Plus

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- 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 Amplifier. Incubate for 10 minutes
- Rinse slides in two changes of buffer.
- Tap off excess buffer; apply HRP Polymer. Incubate for 10 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, Mayer Modified (Part 1202) for 2 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:

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|--------------------------|------------------------|
| 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 staining 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 (CM220A) is the concentrated primary antibody used and diluted to 1/100 working dilution.
- Biocare MSH2 (CM219A) is the concentrated primary antibody used and diluted to 1/400 working dilution.
- Biocare MSH6 (CM265A) is the concentrated primary antibody used and diluted to 1/250 working dilution.
- Biocare PMS2 (CM334A) is the concentrated primary antibody used and diluted to 1/200 working dilution.
 - Antibodies are diluted with Cell Marque Emerald: Antibody Diluent (936B).
- Cell Marque HiDef Detection[™] HRP Polymer System (954D) provides the Amplifier and HRP Polymer solutions 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.
- Cell Marque Emerald: Antibody Diluent datasheet.
- Cell Marque HiDef Detection[™] HRP Polymer System datasheet.
- Cell Marque DAB Substrate Kit datasheet.
- Modifications developed by Newcomer Supply Laboratory.