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Part 3237
Revised December 2016

Cytokeratin, HMW Control Slides – Technical Memo

CONTROL SLIDES: **Part 3237A** 10 Slide/Set **Part 3237B** 98 Slide/Set

Cytokeratin, HMW Control Slides contain sections of positive staining skin and negative staining myometrium.

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 serial section within the control box is stained and provided for your reference. ***Before using the unstained slides, review the enclosed stained slide 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 one year from the date of receipt. Revalidate after one year 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 Cytokeratin, HMW (High Molecular Weight) Control Slides are for the positive immunohistochemical staining of Cytokeratin, HMW, expressed in squamous, ductal and complex epithelia. Useful in differentiation of benign prostate glands from prostatic adenocarcinoma and for the classification of neoplastic tissue as carcinoma or epithelial origin.

METHOD:

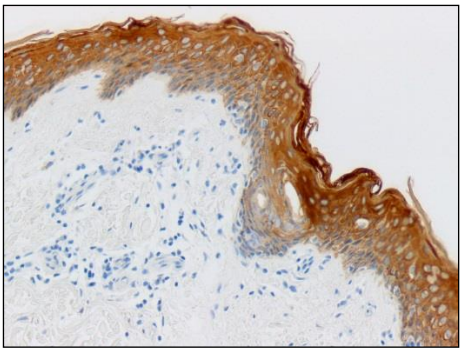
Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns on Superfrost® Plus

NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. 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.*
2. Proceed, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
3. Rinse in distilled water; tap off excess water.
4. Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
5. Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
    a. *See Procedure Note #2.*
6. Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
    a. *See Procedure Note #3.*
7. Tap off excess buffer; apply Cytokeratin, High Molecular Weight primary antibody. Incubate at room temperature for 30 minutes.
8. Rinse slides in two changes of buffer.
9. Tap off excess buffer; apply Link. Incubate for 10 minutes.
10. Rinse slides in two changes of buffer.
11. Tap off excess buffer; apply Label. Incubate for 10 minutes.
12. Rinse slides in two changes of buffer.
13. Prepare required quantity of DAB substrate/chromogen.
14. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
15. Rinse slides in four changes of distilled water.
16. Counterstain lightly with Hematoxylin Stain, Mayer Modified (Part 1202) for 2 minutes.
17. Rinse slides in warm tap water to blue sections.
18. 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:

Cytokeratin, HMW positive expression	Brown cytoplasmic staining
Myometrium	Negative
Nuclei	Blue



PROCEDURE NOTES:

1. Do not allow sections to dry out at any point during staining 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. Agilent Cytokeratin, High Molecular Weight (34ßE12) is the pre-dilute primary antibody used.
5. Agilent LSAB2 Visualization Kit provides the Link and Label solutions used.
6. Cell Marque DAB Substrate Kit is the chromogen used.
7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Agilent Cytokeratin, High Molecular Weight Antibody datasheet.
2. Agilent LSAB2 Visualization Kit datasheet.
3. Cell Marque DAB Substrate Kit datasheet.
4. Modifications developed by Newcomer Supply Laboratory.

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