

Cytokeratin, HMW Control Slides – Technical Memo

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

PRODUCT SPECIFICATIONS:

Tissue: Positive staining skin and negative staining myometrium.

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

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: Cytokeratin, HMW quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year 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 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 classification of neoplastic tissue as carcinoma or epithelial origin.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

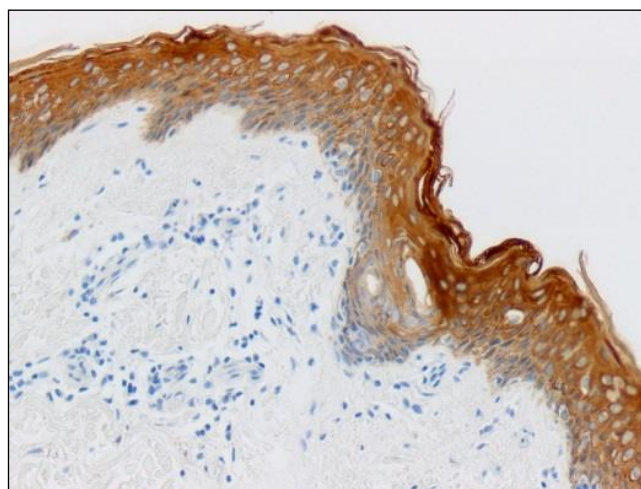
1. Heat dry sections in oven according to your laboratory protocol.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Note #1.
3. Proceed, 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 Cytokeratin, High Molecular Weight primary antibody. Incubate at room temperature for 30 minutes.
9. Rinse slides in two changes of buffer.
10. Tap off excess buffer; apply Link. Incubate for 10 minutes.
11. Rinse slides in two changes of buffer.
12. Tap off excess buffer; apply Label. Incubate for 10 minutes.
13. Rinse slides in two changes of buffer.
14. Prepare required quantity of DAB substrate/chromogen.
15. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
16. Rinse slides in four changes of distilled water.
17. Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
18. Rinse slides in warm tap water to blue sections.
19. 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.

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. Agilent Cytokeratin, High Molecular Weight (34βE12) 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.



RESULTS:

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