

Cytokeratin 5/6 (CK5/6) Control Slides – Technical Memo

CONTROL SLIDES: **Part 3230A** **Part 3230B**
10 Slide/Set 98 Slide/Set

Cytokeratin 5/6 (CK5/6) Control Slides contain sections of positive staining tonsil and negative staining liver.

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 5/6 (CK5/6) Control Slides are for the positive immunohistochemical staining of CK5/6, a useful marker in identification of mesothelioma and lung squamous cell carcinoma and also expressed in a wide range of normal tissue such as breast, prostate, mesothelium, skin and esophagus.

METHOD:

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

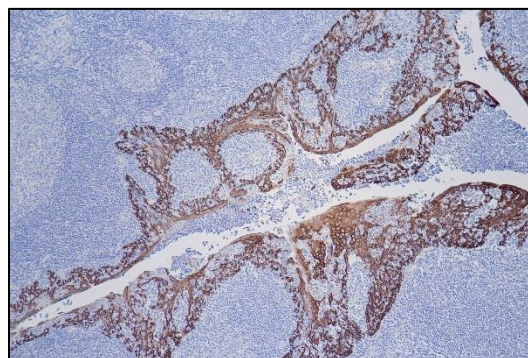
Technique: Paraffin sections cut at 4 microns on Superfrost® Plus

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 CK5/6 primary antibody. Incubate at room temperature for 30 minutes.
8. Rinse slides in two changes of buffer.
9. Tap off excess buffer; apply Amplifier. Incubate for 10 minutes.
10. Rinse slides in two changes of buffer.
11. Tap off excess buffer; apply HRP Polymer. 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, Gill I (Part 1180) for 5 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:

CK5/6 positive expression	Brown cytoplasmic staining
Liver	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. Cell Marque CK5/6 (D5/16B4) is the concentrated primary antibody used. Dilute primary antibody to 1/200 working dilution with Cell Marque Emerald: Antibody Diluent (936B).
5. Cell Marque HiDef Detection™ HRP Polymer System (954D) provides the Amplifier and HRP Polymer solutions used.
6. Cell Marque DAB Substrate Kit (957D) is the chromogen used.
7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Cell Marque CK5/6 Antibody datasheet.
2. Cell Marque Emerald: Antibody Diluent datasheet.
3. Cell Marque HiDef Detection™ Polymer System datasheet.
4. Cell Marque DAB Substrate Kit datasheet.
5. Modifications developed by Newcomer Supply Laboratory.