CYTONEL-PLUS

ULTRARAPID IHC KIT

CNP-20 / CNP-100

CE
IVD

INTRODUCTION

The Cytonel-Plus kit offers reagents for rapid pan-cytokeratin immunostaining. The kit includes a peroxidase-conjugated pan-cytokeratin antibody, which is optimised for the ultrarapid staining protocol. The antibody is detected using diamino benzidine (DAB) chromogen. The staining protocol takes ~ 6 min to perform.

CYTONEL-PLUS KIT CONTENT (20 test and 100 test available)

- **Endogenous peroxidase blocker**
  Refer to Material Safety Data Sheet for additional information.
- **Peroxidase-conjugated Fab2 fragment of a pancytokeratin antibody (ready to use)**
  The antibody reacts with an epitope which is present in cytokeratins 4, 5, 6, 8, 10, 13, 18, and 19.
- **Negative control antibody reagent (ready to use)**
- **DAB chromogen concentrate**
  Please note, DAB is a suspected carcinogen. Avoid contact with skin and clothing. Dispose waste in accordance with local regulations. Refer to Material Safety Data Sheet for additional information.
- **Diamino benzidine (DAB) chromogen and dilution buffer**
- **PBS-tween wash buffer tablet (dissolve in 1 L dH_2O)**

Also needed:

- Thermal plate +42 °C (± 2 °C)
- Slide spinner (e.g. Labnet slide spinner) optional
- Distilled or deionized water
- Acetone
- Hydrogen peroxide, E.g. Sigma Cat no: H1009
- Hematoxylin working solution
- Coplin jars, coverslips, pipette.
- Mounting medium: Cytonel-Plus staining is compatible with aqueous and xylene based mounting.

For aqueous mounting we recommend to use e.g. VectaMount AQ (from Vector Laboratories) or Faramount (Dako). Aqueous mounting medium can be applied immediately after washing away the excess of counterstain.

For permanent mounting we recommend to dehydrate the slides with 70 %, 94 % and absolute ethanol (10 sec. each), followed by xylene (20 sec) and xylene-based mounting medium (Pertex, Permount etc.)

Store reagents at +2 °C to +8 °C.

PREPARATION OF CONTROL SLIDES

- Cut frozen sections from freshly frozen tissues known to contain keratin-positive epithelial cells. Prostate and appendix are well-suited for this purpose.
- Pick up the sections on the microscope slides using laboratory's normal procedure.
- Airdry for 2 min and store the slides in a freezer at -20 °C until used. The slides are usable for 2-4 weeks. Control slide use: Take a control slide from the freezer and fix together with the test slide. Perform all subsequent staining steps as indicated in the working protocol.

RECOMMENDED PREPARATION OF SENTINEL NODE FOR FROZEN SECTIONING

See Krogerus L et al.: Towards reasonable workload in diagnosis of sentinel lymph nodes: comparison of two frozen section methods. Histopathology 2004, 44:29-34.

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PREANALYTICAL STEPS

Prepare fixative: Add 1 ml of 3 % hydrogen peroxide (H_2O_2) and 0.5 ml of endogenous peroxidase blocker (included in the kit) in 100 ml of commercial grade acetone. The fixative is usable for 6 hours.

Prepare DAB working solution: Add 15 microliters of DAB chromogen to 0.5 ml of DAB diluent and mix well. The DAB working solution is usable for up to 2 days when stored at +4 °C after initial preparation. 0.5 ml is sufficient for staining of two slides.

CYTONEL-PLUS KIT WORKING PROTOCOL

1. **Cut cryosections according to laboratory's standard procedure. Let the slide to airdry for 1-2 min at room temperature.**
   It is recommended to include a control slide together with the test slide from this step onwards (see preparation of control slides).
2. **Fix slide in acetone- 0.03 % H_2O_2-peroxidase blocker for 2 min (±20 sec) at room temperature. Let slide to airdry.**
   NOTE: Endogenous peroxidase activity is eliminated during this step.
3. **Place slide on a thermal plate (42±2 °C) and apply anti-pancytokeratin-HRP antibody conjugate (ready-to-use). Incubate for 3 min (±10 sec).**
4. **Wash slide in PBS-tween (agitate slide manually for 10 seconds).**

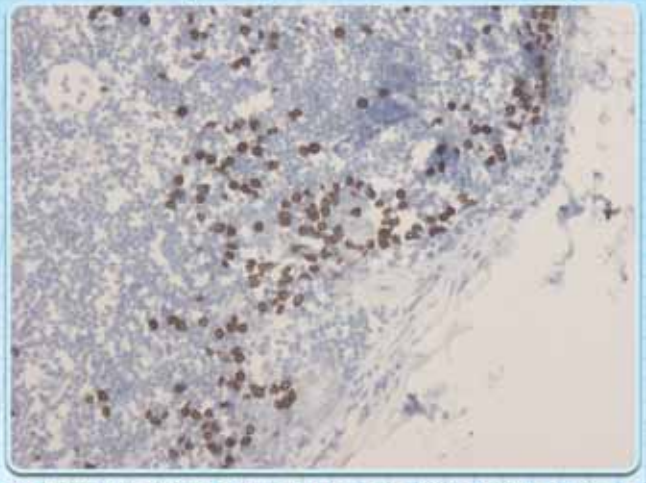
5A. Blot to dry. Make sure that slide is dry before applying DAB.

5B. Optional: Centrifuge slide for 10 sec. in slide spinner to dry.

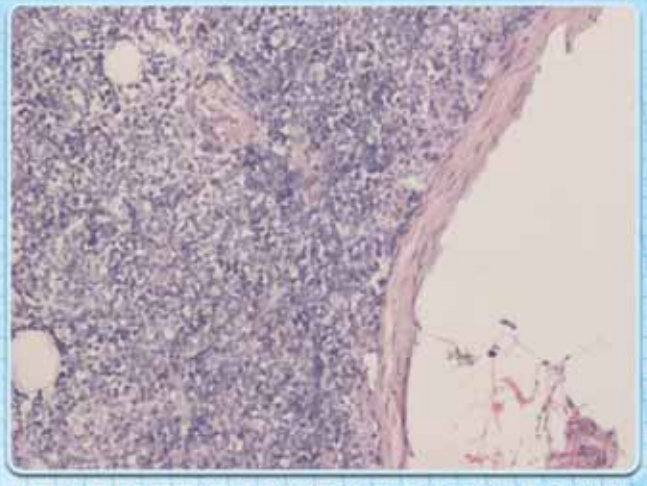
6. **Place slide on thermal plate (42±2 °C) and apply DAB working solution. Incubate for 1.5 min (±10 sec).**
7. **Wash slide in PBS-tween for 5-10 sec.**
8. **Counterstain with hematoxylin (5-10 sec), wash, and mount using laboratory's standard procedure. Both aqueous and permanent mounting can be used.**

STAINING INTERPRETATION

Brown DAB precipitate localizes the cytokeratin-positive cells



A frozen section of a sentinel node stained with Cytonel-Plus. Metastatic lobular carcinoma cells are identfied as brown.



An adjacent section stained with H&E. Note the difficulty to identify carcinoma cells.

For troubleshooting, see troubleshooting guide at www.jilab.fi/cytonelplus

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