ihcDirect® polyHRP Staining Kit: -Anti Human Pan-CK (Clones AE1/AE3/C94/R226)

Catalog Number & Package Size: RK41058-010: For 50-100 IHC Tests with frozen or FFPE tissues
RK41058-005: For 25-50 IHC Tests with frozen or FFPE tissues

Concentration: Ready to use

Storage: Store at 4°C upon receiving. **DO NOT FREEZE.**

Intended Use: For Research Use Only.

This product is designed and developed to meet the need for ultra-fast immunohistochemistry (IHC) testing at convenient room temperature. The assay is optimized to work on frozen tissue sections using a 10 minute IHC protocol.

Introduction: The key component in this kit is horseradish peroxidase (HRP) polymer (PolyHRP) labeled anti-human cytokeratin antibodies, clones AE1, AE3, C94 and R226. This four antibody cocktail well addressed the weak or none staining issue of classical AE1/AE3 cocktail on multiple frozen tissues. The product was developed based on using Novodiax's proprietary enzyme polymerization technology and Direct IHC technology called ihcDirect (Patent Pending) and is optimized for IHC on frozen tissues. The incubation time on frozen tissues is 3 minutes and on FFPE tissues 15-30 minutes. Cytokeratins are cell lineage marker for epithelial cells and can be used for detection of cells of epithelial origins.

Kit Components:

Components	RK41058-010	RK41058-005
PolyHRP labeled anti-human cytokeratin antibodies, clones AE1, AE3, C94 and R226 cocktail in a dropper bottle (ready to use)	10 ml	5.0 ml
Common IHC Blocker in a dropper bottle (ready to use)	10 ml	5.0 ml
DAB Reaction Solution	10 ml	5.0 ml
DAB Chromogen Solution	300 µl	150 µl

Common Histology Reagents and Materials Required but Not Provided:

(1) A slide warmer that can be set at 30°C (optional).
(2) Frozen tissue fixative solution (25 ml of glacial acetic acid, 100 ml of 37% formaldehyde, 375 ml of methyl alcohol, alternatively **cold acetone**)
(3) Hematoxylin
(4) Xylene
(5) Ethanol
(6) Aqueous mounting media (such as VectaMount™ AQ Mounting Medium)
(7) PBS-T
(8) Cover slips
(9) Pipettor

www.novodiax.com
Sales@novodiax.com
Support@novodiax.com

Phone: +1 (510) 342-3043
Fax: +1 (510) 254-6704
3517 Breakwater Ave
Hayward, CA 94545
U.S.A.

(10) Timer
(11) Light microscope
(12) Antigen retrieval buffer and instrument if using FFPE tissues

Staining protocol for frozen tissues

Pre-warm all reagents to room temperature prior to use.

Shake the PolyHRP-labeled antibody solution **gently** before use. **Do not vortex.**

Novodiax K50001-030 DAB chromogen kit is validated for this protocol. Calculate how much DAB solution is needed (200 µl per slide) and **freshly** prepare DAB working solution by adding 30 µl of DAB Chromogen Solution to 1 ml of DAB Reaction Solution.

The following protocol was validated at a room temperature between 21°C-30°C (70°F-86°F) as well as on a slide warmer set at 30°C. If your room temperature is lower than 21°C, a longer incubation time (≥5 minutes depending upon temperature) with PolyHRP labeled antibody is required. To obtain consistent result, a slide warmer set at 30°C is strongly recommended for incubations with Blocker, PolyHRP-labeled antibody and DAB working solution.

Frozen tissue sectioning and fixation: Freshly sectioned tissue must be immediately fixed in Fixative solution for at least 30 seconds.

A 10 minute IHC protocol for frozen tissue sections

Procedure	**Time in minute**
Wash twice with PBS-T	0.25
Blocking with Common IHC Blocker	**1**
Remove the Blocker by dabbing on tissue paper, no wash	
PolyHRP -Antibody	**3**
Wash twice with PBS-T	0.25
DAB working solution	**1-3**
Wash twice with PBS-T or tap water	0.25
Hematoxylin counterstaining	**0.25**
Wash twice with PBS-T or tap water	0.25
Counter Staining + mounting medium + coverslip	1
Total	**10**

Staining protocol for FFPE tissues

- Deparaffinization: 3X 5 minutes in Xylene, then 3 minutes each in 100%, 95% and 75% ethanol. Then wash slides with tap water in slide tank for two times, 2 min each time.
- Antigen retrieval: Using a water bath, incubate slides in antigen retrieval buffer (10mM Citric acid, pH 6.0, 0.02% Tween 20) in a slide tank at 96°C for 30 min, then cool the slides down to room temperature for 30 minutes. Rinse the slides twice with tap water, 2 min each time.
- (Optional) Quench with H_2O_2: Soak the slides in 3% H_2O_2 in slide tank, stand for 10 min. Rinse the slides with tap water twice and then wash once with PBS-T in slide tank for 2 min.

- Add 200 µl of Common IHC Blocker and incubate at room temperature for 15 min. Rinse the slides twice with PBS-T in slide tank, 2 min each time.
- Load 200µl of PolyHRP labeled AE1/AE3 antibody cocktail onto the slides and incubate for 15 minutes at room temperature. Rinse the slides twice with PBS-T in slide tank, 2 min each time.
- Add DAB working solution, incubate for 3-5 minutes at room temperature. Rinse the slides twice with tap water in slide tank, 2 min each time.
- Counterstaining: Add hematoxylin and incubate for 1 min at room temperature. Rinse twice with tap water, 2 min each time.
- Dehydration: Soak slides in the following order: 75% ethanol for 3 min, 100% ethanol for 3 min, Xylene for 3x 5 min.
- Coverslipping: Add one drop of permanent mounting medium on each slide, place a coverslip.

Expected results: Intense brown color stains with clean background if cytokeratin-expression cells exist. No brown color stains if no cytokeratin-expression cells exist.

Unexpected results and troubleshooting:

Frozen tissues:

- No staining: If no staining on positive control slide, please verify whether (1) chromogen was prepared freshly and correctly, (2) reagents were used in right order, (3) PolyHRP-labeled antibody was indeed added, then repeat the procedure.
- Weak staining: Please check whether (1) the reagents have expired, (2) room temperature was below 21°C if a 30°C slide warmer was not used, (3) chromogen was prepared freshly, and (4) too much washing solution remained on slide and diluted next reagent, then take corrective action and repeat the procedure.
- High background: Possible causes include (1) inadequate washes, (2) blocker was ignored, (3) specimens dry out, (4) prolonged incubation with chromogen, (5) prolonged incubation with PolyHRP-labeled antibody and (6) specimens contain high level of endogenous peroxidase and need an additional blocking step (please refer to the Quench with H_2O_2 step in “Staining protocol for FFPE tissues” in previous page. Please repeat the procedure after a cause is identified.

FFPE tissues:

- If unexpected "no staining" or "weak staining" is observed, please verify whether dewaxing and antigen retrieval were performed adequately in addition to check the potential causes above for frozen tissues then repeat the procedure.

Should a trouble remains after all of above troubleshooting actions have been taken, please contact our technical support department immediately.

Trademark: ihcDirect®

Patent: Direct immunohistochemistry technology (Patent pending, PCT/US2015/024388)