NOVODIAX

Advancing immunoassays through innovation

ihcDirect® Cytokeratin 8/18 Kit
Anti-Human Cytokeratin 8/18 (Clone C94)

K41001-010 100 tissue stains*
K41001-005 50 tissue stains*

Intended Use: For In Vitro Diagnostic Use

Polymerized horseradish peroxidase (pHRP)-labeled anti-human cytokeratin 8/18 (CK 8/18) antibody (cytokeratin 8/18 pHRP) is intended for laboratory use to qualitatively identify by light microscopy the presence of CK 8/18 in sections of formalin-fixed, paraffin-embedded tissue sections or frozen tissues using immunohistochemistry (IHC) test methods. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist/physician. This conjugate has been pre-diluted and optimized for IHC use without further dilution.

Summary and Explanation:

The Cytokeratin 8/18 antibody recognizes human cytokeratin intermediate low molecular weight filament proteins of 52.5 kD and 45 kD, i.e., cytokeratins 8 and 18, respectively. Cytokeratins 8 and 18 are expressed in most simple and glandular epithelium, such as thyroid, female breast, gastrointestinal tract, and respiratory tract. In cancer tissues, adenocarcinomas express these proteins, but keratinizing squamous cell carcinomas do not. Antibodies to CK8 and CK18 may be utilized in the classification of tumors of epithelial origin (Cimpean et al., 2008; Reisenblichler 2013). The key component in this kit is horseradish peroxidase (HRP) polymer (PolyHRP) labeled mouse anti-human cytokeratin 8/18 antibody (clone C94). The ihc Blocker provided in the kit used prior to applying CK 8/18 pHRP conjugate will reduce background and nonspecific staining. Chromogen 3,3'diaminoben-zidine (DAB) is used in this kit.

Principle of Procedure:

The ready-to-use ihcDirect Cytokeratin 8/18 antibody pHRP conjugate is directly applied to pretreated tissue sections, where it binds to Human CK 8/18. A DAB working solution is then applied to the tissue. The CK 8/18 antibody-linked pHRP catalyzes the DAB to form a visible brown color product which precipitates at the location of human cytokeratin 8/18. The specimen may then be counterstained with hematoxylin and a coverslip applied. Results are viewed and interpreted using a light microscope. Volumes are based upon 100 µl antibody per tissue. This IHC kit may be performed either manually or on an open automatic IHC staining system.

Reagents Provided:

Kit Part No.	Σ	Description
K41001-005*	50*	5ml size ihcDirect CK 8/18 ready-to-use antibody conjugate, ihc Blocker and equivalent volumes of ihc DAB and ihc DAB Diluent.
K41001-010*	100*	10ml size ihcDirect CK 8/18 ready-to-use antibody conjugate, ihc Blocker and equivalent volumes of ihc DAB and ihc DAB Diluent.

* At estimated volume of 100 µl of antibody conjugate per tissue

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Human Cytokeratin 8/18	C94	Mouse	IgG	10 mg/ml

CK 8/18 antibody is a mouse monoclonal antibody to human cytokeratin 8/18 purified from ascites. HRP is extracted from horseradish plant. The ihc Blocker contains normal goat serum and 1% BSA in a proprietary buffer system with 0.01% Thimerosal as preservative.

The ihc DAB Diluent contains the buffered peroxide substrate. The ihc DAB (chromogen) contains 3,3'diaminobenzidine which is dissolved in a proprietary buffer system with no hazardous chemicals at a reportable concentration. This reagent is light sensitive. For best results, minimize the time the vial is open. Keep away from light.

Cytokeratin 8/18 pHRP Kit Components for 5ml and 10ml Test Kits:

Kit Components	Component Part Numbers	Sizes
CK8/18 pHRP	H31001-(R###) (005, 010)	5ml, 10ml
ihc Blocker	C30005-(###ML) (005, 010)	5ml, 10ml
ihc DAB Diluent	C30004-(###ML) (007, 013)	7ml, 13ml
ihc DAB	C30003-(###UL) (200, 375)	200µl, 375µl

Materials Needed but Not Provided:

The following reagents/supplies may be required in staining but are not provided:

1. Frozen section tissue fixative (ihc Fixative) or reagent grade acetone
2. Positive and negative control tissues
3. Microscope slides, positively charged (required)
4. Staining jars, baths or processing tools
5. ihc Wash Buffer (PBS-T)
6. Pipettor and pipet tips
7. Timer
8. Antigen retrieval buffer (when using FFPE tissues)
9. Peroxide blocker (optional)
10. Instruments used for tissue pretreatment, such as water bath, or pressure cooker or microwave oven (when using FFPE tissues)
11. Hematoxylin
12. Xylene or Xylene substitute
13. Ethanol
14. Mounting medium
15. Cover slips
16. Light microscope (40 - 400x)

Novodiax Bulk Reagent Formulations:

1. ihc Fixative, (375ml of methyl alcohol, 100ml of 37% formaldehyde and 25ml of glacial acetic acid).
2. ihc Wash Buffer (PBS-T), (10 mM phosphate buffer, pH7.2, 150 mM NaCl, 0.05% Tween-20).
3. ihc Antigen Retrieval Buffer (10mM Citric buffer, pH 6.0, 0.02% Tween 20).

Storage and Handling:

The kit should be stored at 2-8°C. Do not freeze. DAB working solution should be made prior to use and is stable at 2-8°C during the day the reagents are made. This kit is suitable for use until expiry date when stored at 2-8°C. Do not use the product after expiration date stamped on vial unless dating extension information is provided by Novodiax. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.

Specimen Preparation:

Paraffin Sections: Tissues routinely processed, neutral buffered 10% formalin-fixed are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980). Variable results may occur as a result of prolonged fixation. Each section should be cut to the appropriate thickness (approximately 4-5 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least one hour but not exceeding 24 hours in a 58-60°C±5°C oven. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades (Kiernan, 1981; Sheehan & Hrapchak, 1980).

Frozen Tissue Sections: Frozen tissue is sectioned to the appropriate thickness (approximately 5 µm) and placed on a positively charged glass slide. Tissues should be fixed in either the Novodiax ihc Fixative or reagent grade acetone for 30-seconds-to-1-minute immediately after sectioning. Reagent grade acetone may be kept cold, e.g. at cryostat temperatures or at room temperature. Following fixation, tissues may be stored in PBS-T for as long as a day.

Treatment of Tissues Prior to Staining: Pretreatment is tissue dependent and should be performed as suggested in the staining procedure sections.

Warnings and Precautions:

1. The CK8/18 antibody-pHRP conjugate is pre-diluted. Further dilution may reduce signal intensity or produce false-negative staining. These recommendations are for guidance only. Laboratory managers should determine their own procedures and quality policies.
2. Take reasonable precautions when handling reagents. Use protective equipment such as disposable gloves and lab coats when handling suspected carcinogens or toxic materials. Read Safety Data Sheets (SDS) prior to use.
   a. Thimerosal is used as a preservative in this solution and the substance is classified as a toxic substance. Inhalation causes respiratory and CNS effects and severe delayed neurotoxicity.
   b. WARNING! DAB product contains 3,3'diaminobenzidine which may cause genetic effects and/or cancer. If exposed or concerned, seek medical attention. See DAB SDS for more information.
3. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
4. Use charged slides to secure tissue adhesion.
5. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
6. Consult local or state authorities with regard to recommended methods of disposal of bio-hazardous and hazardous chemical waste materials.
7. Incubation time and temperature other than those specified may give erroneous results. The user must validate any such changes.
8. Use lab grade quality chemicals such as acetone, ethanol and water when preparing fixatives and buffers. Users should validate performance including stability for laboratory prepared reagents (at 1X).
9. Avoid microbial contamination of reagents.

Staining Procedures:

General Operating Notes:

1. Equilibrate all reagents to room temperature prior to use. Swirl or shake the pHRP-labeled antibody solution before use. **Do not vortex.** Calculate the amount of DAB working solution needed (100 µl per tissue) and **freshly** prepare DAB working solution by adding the ratio of 30 µl of ihc DAB to 1.0 ml of ihc DAB Diluent into a microcentrifuge tube.
2. It is best to prevent slides from drying out during the staining process to avoid unwanted background staining.
3. Gently and thoroughly wash tissues during manual wash steps. Avoid direct high velocity streams of wash that might tend to damage or cut delicate tissues.
4. Following each manual assay step, remove excess fluids on tissue slides with tissue paper. Excessive residual solution may dilute subsequent reagents, causing negative or uneven staining.
5. For the tissues with high levels of oxidase activity, e.g. gastrointestinal or renal tissues, an additional blocking step with H_2O_2 is required to minimize background.
6. The following protocol for frozen tissues has been validated at temperatures between 21°-30°C (70°- 86°F) for incubating ihc Blocker, CK 8/18 pHRP and DAB working solution. If room temperature is less than 21°C, incubate labeled antibody for a longer period of time (≥4 minutes depending upon temperature). Consistent results have been obtained using a slide warmer set to 30°C.

Frozen Tissue Sections:

1. Place frozen tissue sections into fixation solution immediately after sectioning. **Prolonged exposure to room or freezing temperatures may alter targeted epitopes.**
2. The DAB working solution incubation step is a range from 1-3 minutes. Users should determine the optimal incubation time for their lab environment and observe the brown color formation via visual inspection during incubation.

Test Timing Est. (10-minute IHC protocol for frozen tissue sections):

Procedure Frozen	Time in minutes
Fixation, use Acetone or ihc Fixative	0:30-1:00
- Wash with ihc Wash	0:15
Block with ihc Blocker	1
- Tap and Blot to remove excess blocker	- - -
CK 8/18 pHRP	3
ihc Wash	0:15
- Tap and Blot to remove excess wash buffer	- - -
DAB working solution	1-3
ihc Wash	0:15
Hematoxylin counterstain	0:20
ihc Wash	0:15
Dehydrate/Mount media and coverslip	0:45
Total	**10**

Paraffin Tissues:

1. Deparaffinization: Soak slides in Xylene 3 times for 5 minutes each. Next, 3 minutes each in 100%, 95% and 75% ethanol. Then wash slides with tap water in slide tank for two times, 2 minutes each time.
2. Antigen retrieval: Using a water bath, incubate slides in antigen retrieval buffer in a slide tank at 96°C for 30 minutes, then cool the slides down to room temperature for 30 minutes. Rinse the slides twice with tap water, 2 minutes each time.
3. (Optional) Block tissue with H_2O_2: Soak the slides in 3% H_2O_2 in slide tank, stand for 10 minutes. Rinse the slides with tap water twice and then wash once with PBS-T in slide tank for 2 minutes.
4. Dispense 100 µl of ihc Blocker covering the entire tissue and incubate at room temperature for 15 minutes. Remove ihc Blocker as much as possible but do not rinse the slides with PBS-T or water.
5. Dispense 100µl of pHRP labeled anti-human CK 8/18 antibody on slides covering the entire tissue and incubate for 15-30 minutes at room temperature. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Note: Place slides in a wet chamber to prevent evaporation if longer incubation times are used.
6. Dispense 100µl of DAB working solution covering the entire tissue, incubate for 3-10 minutes at room temperature. Rinse the slides twice with tap water in slide tank, 2 minutes each time.
7. Counterstaining: Add hematoxylin and incubate for 1 minute at room temperature. Rinse twice with tap water for 2 minutes, each time.
8. Dehydration: Soak slides in the following order: 75% ethanol for 3 minutes, 95% ethanol for 3 minutes, 100% ethanol for 3 minutes and Xylene twice at 5 minutes each time.
9. Applying Coverslip: Add one drop of permanent mounting medium on both the slide and the coverslip, then apply coverslip.

Quality Control Procedures:

Positive and negative controls should be run simultaneously with patient specimens.

Positive Tissue Control: The recommended positive control tissues for this antibody are properly processed breast carcinoma and lung adenocarcinoma tissues. The staining is cytoplasmic. One positive tissue control for each set of test conditions should be included in each staining run.

The tissues used for the positive control should be selected from patient specimens with well-characterized low levels of positive target activity that give weak positive staining. Known positive tissue controls should only be utilized for

monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the patient specimens should be considered invalid.

Negative Tissue Control: The same tissue used for the positive control may be used as the negative tissue control. The variety of cell types in most tissue sections offers internal negative control sites. But this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Troubleshooting:

If an unexpected staining pattern occurs on control tissues or patient samples, consider the following:

1. No staining: If no staining on positive control slide, please verify whether (1) chromogen was prepared freshly and correctly, (2) reagents were used in the specified order, (3) pHRP-labeled antibody was indeed added, and (4) for FFPE tissue, dewaxing and antigen retrieval were performed inadequately. Perform any corrective actions required and then repeat the procedure.
2. 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, (4) too much washing solution remained on slide and diluted the next reagent, and (5) for FFPE tissue, dewaxing and antigen retrieval were performed inadequately. Perform any required corrective actions and repeat the procedure.
3. High background: Possible causes include (1) insufficient washes, (2) blocker not applied or washed out after application, (3) specimens dried out, (4) prolonged incubation with chromogen, (5) prolonged incubation with pHRP-labeled antibody and (6) specimens contain high level of endogenous peroxidase and need an additional blocking step (refer to the Block tissue with H_2O_2 step in "Staining Procedures Paraffin Tissues"). Perform any required corrective actions and repeat the procedure.
4. The ihc DAB chromogen volumes provided in the kit are matched to the size of the kit for a typical user. Occasionally, materials can stick to either the lid or the side of the vial. To gain access to all of the material, it may be necessary to centrifuge at a slow speed or tap down the bottle using caution prior to use.

If an unexpected staining pattern is observed on control tissues or patient samples which cannot be explained by variations in laboratory procedures or a problem with the antibody is suspected, contact Novodiax Technical Support or your local distributor immediately. Within the US and Canada call 1 (888) 439-2716 ext. 2 or 1 (510) 342-3043 ext. 2.

Expected Results:

Intense brown color stains with a clean background if cytokeratin 8/18-expression cells exist. No brown color stains if no CK8/18-expression cells exist. Interpretation of the staining result is solely the responsibility of the user.

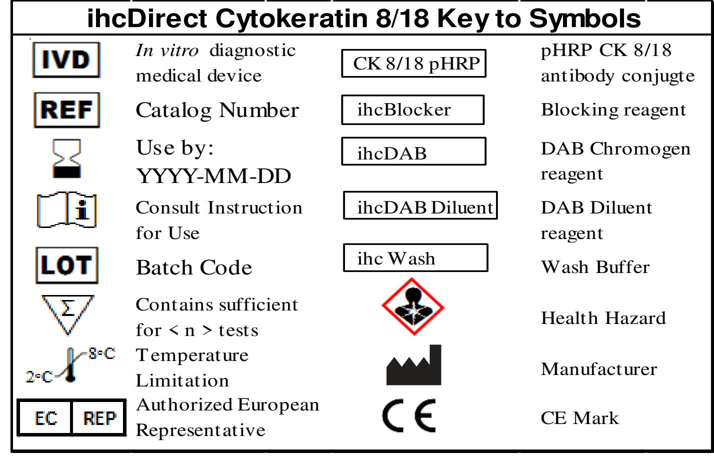
General Limitations:

Immunohistochemistry is a multistep diagnostic process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue (Nadji M, Morales AR. 1983).

The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

Performance Characteristics:

The ihcDirect CK 8/18 pHRP test kit performance has been determined using both frozen and FFPE tissue sections. Novodiax has conducted studies to evaluate the performance of the antibody conjugates, accompanying kit reagents and ancillary supplies. The antibodies and systems have been found to be sensitive and show specific binding to the antigen of interest with minimal to no binding of non-specific tissues or cells. Novodiax antibodies and accompanying kit reagents have shown reproducible and consistent results when used within a single run, between runs and between lots. These products have been determined to be stable for the periods of time specified on the labels either by standard real-time and/or accelerated methods. Novodiax ensures product quality by testing each lot of material and by testing materials at regular intervals and via surveillance programs.



ihcDirect Cytokeratin 8/18 Key to Symbols

Symbol	Meaning	Symbol	Meaning
IVD	*In vitro* diagnostic medical device	CK 8/18 pHRP	pHRP CK 8/18 antibody conjugte
REF	Catalog Number	ihcBlocker	Blocking reagent
	Use by: YYYY-MM-DD	ihcDAB	DAB Chromogen reagent
	Consult Instruction for Use	ihcDAB Diluent	DAB Diluent reagent
LOT	Batch Code	ihc Wash	Wash Buffer
Σ	Contains sufficient for < n > tests		Health Hazard
2°C – 8°C	Temperature Limitation		Manufacturer
EC REP	Authorized European Representative	CE	CE Mark

Instructions for Use (IFU) Access:

To obtain a translation or the latest electronic version of an IFU document, visit our website at https://www.novodiax.com/support/literature/ (ihcDirect IFU). Printed copies of an IFU document may be obtained by contacting Novodiax Technical Support or your local distributor.

Bibliography:

1. Cimpean AM et al. Relevance of the immunohistochemical expression of cytokeratin 8/18 for the diagnosis and classification of breast cancer. Romaniam Journal of Morphology and Embryology. 2008; 49(4):479-483.
2. Reisenblichler ES et al. The predicative ability of a CK5/p63/CK8/18 antibody cocktail in stratifying breast papillary lesions on needle biopsy. Am J Clin Pathol. 2013; 140:767-779.
3. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. New York: Pergamon Press 1981.
4. Sheehan DC and Hrapchak BB. Theory and Practice of Histotechnology. St. Louis: C.V. Mosby Co. 1980.
5. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med, 1983; 14:767-771.

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

Novodiax, Inc.
3517 Breakwater Ave
Hayward, CA 94545, USA
Phone: +1 (888) 439-2716

EC REP
EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands