NOVODIAX

Advancing immunoassays through innovation

ihcDirect® CD45 Ab
Anti-Human Cluster of Differentiation (Clone C95)

K31010-015 150 tissue stains*
K31010-010 100 tissue stains*
K31010-005 50 tissue stains*

Intended Use: For In Vitro Diagnostic Use

Polymerized horseradish peroxidase (polyHRP)-labeled anti human CD45 (Clone C95) antibody is intended for laboratory use to qualitatively identify by light microscopy the presence of CD45 molecules which are expressed in hematopoietic cells or tumors in sections of formalin-fixed, paraffin-embedded (FFPE) and/or frozen tissues (FT) using immunohistochemistry (IHC) test method. 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 and proper controls interpreted by a qualified pathologist and/or physician. This conjugate has been pre-diluted and optimized for IHC use without further dilution.

Summary and Explanation:

The ihcDirect® CD45 is a polyHRP conjugated mouse monoclonal antibody intended for laboratory use as a qualitative immunohistochemistry reagent for the identification of a broad range of leucocytes. CD45 is also named Leucocyte Common Antigen (LCA). It expresses in all leucocytes including both B and T lymphocytes as well as other hematopoietic cells, such as basophils, granulocytes, macrophages / histiocytes, mast cells, and monocytes. This test may be used on frozen human tissues (FT) and formalin-fixed-paraffin-embedded (FFPE) human tissues and applied to individuals suspected of having related forms of lymphomas, other hematopoietic tumors, etc.

Principle of Procedure:

The ready-to-use ihcDirect poly HRP CD45 antibody conjugate is directly applied to pretreated tissue sections, where it binds to human CD45. A DAB working solution is then applied to the tissue. The CD45 antibody-linked pHRP catalyzes the DAB to form a visible brown color product which precipitates at the site of the human CD45 location. 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 reagent may be performed either manually or on an open automatic IHC staining system. Please read all procedures and Warmings and Precautions sections prior to use.

Reagents Provided:

Part No.	Σ	Description
K31010-015*	150*	15ml of ihcDirect CD45 ready-to-use antibody conjugate for use with DAB Kit and ihc Blocker.
K31010-010*	100*	10ml of ihcDirect CD45 ready-to-use antibody conjugate for use with DAB Kit and ihc Blocker.
K31010-005*	50*	5ml of ihcDirect CD45 ready-to-use antibody conjugate for use with DAB Kit and ihc Blocker.

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

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Human CD45	C95	Mouse	IgG	10 mg/ml

CD45 antibody is a mouse monoclonal antibody to human CD45 purified from animal origin-free cell culture supernatant. HRP is extracted from horseradish plant. This assay is designed for use with Novodiax ihc Blocker and DAB Kit reagents.

CD45 pHRP reagent components for 5ml, 10ml and 15ml volumes:

Reagent Description	Component Part Numbers	Sizes
CD45 pHRP	H31010-(R###) (005, 010, 015)	5ml, 10ml, 15ml
For Use With Novodiax Antibodies:		
ihc Blocker Intl. USA	K51001-(015) (OR) K51002-(015)	15ml 15ml
DAB Kit	K50001-(###), (015, 030)	15ml, 30ml

Materials Needed but Not Provided:

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

1. Frozen section 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)

* For FFPE tissues only

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, pH 7.2, 150 mM NaCl, 0.05% Tween-20).
3. ihc Antigen Retrieval Buffer (10 mM Citric buffer, pH 6.0, 0.02% Tween 20).

Storage and Handling:

The reagent should be stored at 2-8°C. Do not freeze. This reagent 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 the reagent is stored under any conditions other than those specified in the package insert, they must be verified by the user. DAB working solution should be made just prior to use and is stable at 2-8°C during the day the reagents are made.

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 CD45 pHRP antibody conjugate is pre-diluted. Further dilution may reduce signal intensity or false-negative staining. These recommendations are for guidance only. Laboratory managers should determine their own procedures and quality policies.
2. When performing the assay with frozen tissues, do not use permanent mounting media. Use aqueous mounting media only.
3. Read and understand all of the Novodiax Instructions for Use (IFUs) before product use.
4. For best results, minimize the time the DAB vial is open. This reagent is light sensitive; store the mixed working solution of ihc DAB and ihc DAB Diluent away from light. Refrigerate when not in use.
5. 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 ihc Blocker and the substance is classified as 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.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Use charged slides to secure tissues appropriately.
8. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
9. Consult local or state authorities with regards to recommended methods of disposal of bio-hazardous and hazardous chemical waste materials.
10. Incubation time and temperature other than those specified may give erroneous results. The user must validate any such changes.
11. 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).
12. Avoid microbial contamination of reagents.

Staining Procedures:

General Operating Notes:

1. Equilibrate all reagents to room temperature prior to use. **S**wirl 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 an Eppendorf 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 level 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 has been validated at temperatures between 21°-30°C (70°-86°F) for incubating ihc Blocker, CD45 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	Start
- Wash with ihc Wash Buffer	0:15
Block with ihc Blocker	1
- Tap and Blot to remove excess blocker	- - -
CD45 pHRP	3
- Wash with ihc Wash Buffer	0:15
- Tap and Blot to remove excess wash buffer	- - -
DAB working solution	1-3
- Wash with ihc Wash Buffer	0:15
Hematoxylin counterstain	0:20
- Wash with ihc Wash Buffer	0:15
Aqueous mounting 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 CD45 antibody on slides covering the entire tissue and incubate for 15-20 minutes at room temperature. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Note: Places 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 known CD45 positive tissues. One positive tissue control for each set of test conditions should be included in each staining run. Previous tissue specimen and in some cases, an individual's own tissue may be used as a control.

The tissues used for the positive control should be selected from patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. Known positive tissue controls such as tonsil or lymph nodes 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 (false positive staining) occurs in the negative tissue control, results with the patient specimens should 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 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 washing, (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 volume provided in the DAB Kit is based on 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 CD45-expression cells exist. No brown color stains if no CD45-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 CD45 pHRP test performance has been determined using both frozen and FFPE tissue sections. Novodiax has conducted studies to evaluate the performance of the antibody conjugate, separate Novodiax 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. The Novodiax antibody and separate 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 CD45 Key to Symbols

IVD	*In vitro* diagnostic medical device	CD45 pHRP	pHRP CD45 antibody conjugte
REF	Catalog Number		Manufacturer
	Use by: YYYY-MM-DD	CE	CE Mark
	Consult Instruction for Use	2°C 8°C	Temperature Limitation
LOT	Batch Code	Σ	Contains sufficient for <n> tests
EC REP	Authorized European Representative		

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.

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