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Advancing immunoassays through innovation

ihc CD34 Ab
(Clone QBEND/10)

Ab: KF31046-005, 50 tissue stains*
Ab: KF31046-003, 30 tissue stains*

Intended Use: For *In Vitro* Diagnostic Use

ihc CD34 Ab (Clone QBEND/10) is intended for laboratory use to qualitatively identify by light microscopy the presence of the marker on hematopoietic progenitor cells and endothelial cells 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. These reagents have been formulated to ready-to-use concentration and optimized for IHC on specific tissue application use without further dilution.

Summary and Explanation:

ihc CD34 Ab is a ready to use mouse monoclonal antibody for detecting CD34, a transmembrane glycoprotein expressed on early lymphohematopoietic stem cells, progenitor cells, and endothelial cells. CD34 is highly sensitive for endothelial differentiation, regardless of tumor grade, and recognizes greater than 85% of angiosarcomas and Kaposi's sarcomas. CD34 is so commonly present in dermatofibrosarcoma protuberans (and its variants), spindle cell lipoma, and solitary fibrous tumor that it is regularly used as an adjunct to the diagnosis of these tumors. The ihc Enhancer is following the application of the antibody reagent to add detection system to the antibody binding site. A chromogen such as 3,3'-diaminobenzidine (DAB) is then used to develop color at the reaction site.

Principle of Procedure:

The ready-to-use (RTU) ihc CD34 antibody is applied to pretreated tissue sections, where it binds to CD34 antigens in tissue. Following antibody incubation, tissues are washed and a polymerized horseradish peroxidase (pHRP) labeled detection system (ihc Enhancer) is applied. The tissues are washed again and a Working Solution (WS) of a chromogen such as ihc DAB 1:1 is applied to the tissue. The pHRP on the conjugate reacts with the chromogen to form a visible colored product at the site of CD34 binding location. The specimen may then be counterstained and a coverslip applied. Results are viewed and interpreted using a light microscope. Volumes are based upon 100µl antibody per tissue. This product may be used to perform IHC manually or on an open automated IHC staining system.

Reagents Provided:

Part No.	Σ	Description
KF31046-005*	50*	5ml size ihc CD34 RTU antibody reagent.
KF31046-003*	30*	3ml size ihc CD34 RTU antibody reagent.

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

Clone	Species	Total Protein Conc.
QBEND/10	Mouse	10 mg/mL

CD34 antibody is a mouse monoclonal antibody purified from ascites. HRP is extracted from horseradish plant. The ihc Enhancer is a detection system. Novodiax ihc DAB 1:1 Kit, ihc Magenta 1:1 Kit, are recommended for use with the CD34 antibody reagent.

ihc CD34 Ab Components (K31046-###):

Reagent Description	Component Part Numbers	Sizes (ml)
CD34 Ab	F31046-R### (003, 005)	3, 5

Ancillary Reagents for Use with ihc CD34 Ab:

Reagent Description	Part Numbers	Sizes (ml)
ihc Blocker Intl.	K51001-### (015)	15
ihc Blocker USA	K51002-### (015)	15
ihc Enhancer	K51011-### (015)	15
ihc DAB 1:1 Kit	K50002-### (015, 030)	15, 30
ihc Magenta 1:1 Kit	K50011-### (015, 030)	15, 30

Materials Needed but Not Provided:

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

1. Frozen section fixative (10% NBF§)
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)

§ NBF – neutral buffered formalin

Novodiax Bulk Reagent Formulations:

1. 1X ihc Wash Buffer (PBS-T), (10 mM phosphate buffer, pH7.2, 150 mM NaCl, 0.05% Tween-20) See Part # K52001-800 for 10X concentration buffer.
2. Antigen Retrieval Buffer (10mM Citric buffer, pH 6.0, 0.05% Tween 20).

Storage and Handling:

This product should be stored at 2-8°C and is suitable for use until expiration date when stored at this temperature. Do not freeze. Do not use the product after expiration date 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 validated by the user.

Specimen Preparation:

Paraffin Sections: Tissues routinely processed with 10% NBF 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 reagent grade 10% NBF for 1-2 minutes immediately after sectioning. Reagent grade NBF may be kept cold, e.g. cryostat temperatures, or room temperature. Following fixation, tissues should be processed within a few minutes or may be stored in PBS for several hours.

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. Read and understand all of the Novodiax Instructions for Use (IFUs) before product use.
2. Neutral buffered formalin (NBF) is preferred over acetone for a frozen tissue fixative.
3. The ihc CD34 Ab and ihc Enhancer are pre-diluted. Further dilution may reduce signal intensity or increase the possibility of false-negative staining.

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These recommendations are for guidance only. Laboratory managers should determine their own procedures and quality policies.

4. To obtain best results when working with frozen tissues, it is desirable to freeze tissues as quickly as possible following extraction.
5. Use caution and shorten incubation times when utilizing intense hematoxylin counterstains such as Gills as these stains may tend to mask antibody staining.
6. Take reasonable precautions when handling reagents. Use protective equipment such as disposable gloves and lab coats when handling materials. Read Safety Data Sheets (SDS) prior to use.
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Use charged slides to secure tissue adhesion.
9. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
10. Consult local or state authorities with regard to recommended method of disposal of bio-hazardous and hazardous chemical waste materials.
11. Incubation time and temperature other than those specified may give erroneous results. The user must validate any such changes.
12. Use lab grade quality chemicals such as NBF and water when preparing reagents. Users should validate performance including stability for laboratory prepared reagents (at 1x).
13. Avoid microbial contamination of reagents.
14. Fixation is a vital part of the protocol and fixation times may vary with the fixative chosen, tissue type, e.g. containing fat and other parameters. Generally, an acetone or NBF fixation of 1-2 minutes is recommended. Place frozen tissue sections into fixation solution shortly after sectioning. **Prolonged exposure to room or freezing temperatures may alter targeted epitopes.**
15. It is best to prevent slides from drying out during the staining process to avoid unwanted background staining

Staining Procedures:

General Operating Notes: CD34

1. Equilibrate all reagents to room temperature prior to use. Swirl or shake the ihc Enhancer and antibody solutions before use. **Do not vortex.** Calculate the amount of chromogen WS needed (100µl per tissue) and **freshly** prepare chromogen WS. See instructions for use.
2. 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.
3. 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. Users may also utilize a PAP pen to ensure reagents stay on the desired tissues.
4. To reduce background signal, wash thoroughly following antibody and enhancer step.
5. For the tissues with high oxidase activity, blocking with H_2O_2 may be required to minimize non-specific staining.
6. The following protocol has been validated at temperatures between 21°-30°C (70°-86°F) for incubating antibody reagent and ihc Enhancer and a Novodiax chromogen WS. If room temperature is less than 21°C, users may need to incubate antibody for a longer period of time (≤5 minutes) to achieve satisfactory staining results. Consistent results have been obtained at room temperature or using a slide warmer set to 30°C at the surface of the slide.

Frozen Tissue Sections:

1. Following fixation in NBF, rinse slides with 1x ihc Wash buffer and then wipe away any excess fluid with a Kimwipe® or paper towel.
2. Dispense 100µl of antibody reagent, covering the entire tissue, and incubate for 3 minutes at room temperature. If temperatures are below 21°C manual users may extend incubation times. Then, thoroughly rinse slides with 1x ihc Wash buffer and wipe away any excess fluid.
3. Dispense 100µl of ihc Enhancer, covering the entire tissue, and incubate at room temperature for 3 minutes. Then, thoroughly rinse slides with 1x ihc Wash buffer and wipe away any excess fluid.
4. Dispense 100µl of a chromogen WS, such as DAB covering the entire tissue, and incubate for 1-3 minutes at room temperature. Users should determine the optimal incubation time for their particular chromogen or lab environment. Then rinse slides with either 1x ihc Wash buffer or lab grade water and wipe away any excess fluid.
5. Add a counterstain. Incubation times will vary according to the counterstain formulation. Then, rinse slides with water and wipe away any excess fluid.
6. Apply aqueous media or dehydrate slides with the user's typical dehydration protocol and permanent mounting, then add coverslip.

Test Timing Est. (10-12-minute IHC protocol for frozen tissue sections):

ihc Antibody+Enhancer Frozen Tissue Procedure	Time in minutes
Fix with Acetone or Neutral Buffered Formalin	Start
- Wash with ihc Wash Buffer, remove excess fluid	- - -
**Optional: Block with* ihc Blocker	1
- Tap+Absorb to remove excess blocker	- - -
Novodiax ihc Antibody reagent	3
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess wash buffer	- - -
ihc Enhancer	3
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess wash buffer	- - -
Novodiax Chromogen Working Solution	1-3
- Wash with ihc Wash Buffer or DI water	- - -
Hematoxylin counterstain **(Conc. Dependent)**	2-45 sec
Wash with water	- - -
Dehydrate/Mounting Medium and Coverslip	User Det.
Total	**~10-12**

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. Remove excess fluid.
4. Dispense 100µl of antibody reagent on slides covering the entire tissue and incubate for 10 minutes at room temperature. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Remove excess fluid.
   Note: Place slides in a wet chamber during antibody incubation step to prevent evaporation when longer incubation times are used.
5. Dispense 100µl of ihc Enhancer, covering the entire tissue, and incubate at room temperature for 10 minutes. Then, thoroughly rinse slides with 1x ihc Wash buffer and wipe away any excess fluid.
6. Dispense 100µl of a chromogen WS covering the entire tissue, incubate for 3 minutes at room temperature. Rinse the slides twice with DI or 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 CD34 positive tissues. One positive tissue control for each set of test conditions should be included in each staining run. Previous tissue specimens that have been frozen and freshly cut or in some cases, an individual's own tissue may be used as controls.

The tissues used for the positive control should be selected from patient specimens with characterized positive target activity that gives 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 (false positive staining) occurs in the negative tissue control sites, results with the patient specimens must be considered invalid. Breast carcinoma and lung carcinoma tissues may be used as negative tissue control.

Troubleshooting:

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

1. *No staining*: If no staining is evident on positive control slide, please verify whether (1) chromogen WS was prepared freshly and correctly, (2) reagents were applied in the correct order, (3) the antibody was indeed added, and (4) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any corrective actions required and then repeat the procedure.
2. *Low signal or faint staining*: Please check whether (1) the reagents are not expired, (2) temperature of the testing environment was at least 21°C or a 30°C slide warmer was used, (3) chromogen WS was prepared freshly and correctly, (4) excess ihc Wash solution was not left on the slide, causing subsequent reagents to be diluted, and (5) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any required corrective actions and repeat the procedure. Alternatively, if using a DAB chromogen, consider using another stain, e.g. ihc Magenta 1:1 to obtain more vibrant staining. In addition, some individuals may naturally have low expression of certain antigens. In these cases, users may extend the antibody incubation times by 1-2 minutes.
3. *High background*: Possible causes include (1) insufficient washing, (2) specimens drying out, (3) prolonged chromogen incubation, (4) prolonged antibody incubation, and (5) specimens containing high level of endogenous peroxidase, which necessitates an additional blocking step (refer to the Staining Procedures for Paraffin Tissues). Perform any required corrective actions and repeat the procedure.

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:

Membranous stain. Endothelium acts as a positive internal control. Other types of cells in the same tissue are negative. 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 ihc CD34 Ab test performance has been determined using both frozen and FFPE tissue sections. Novodiax has conducted studies to evaluate the performance of the antibody conjugates, accompanying 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 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.

Key to Symbols

IVD	*In vitro* diagnostic medical device	CD34 Ab	CD34 antibody reagent
REF	Catalog Number		Manufacturer
	Use by: YYYY-MM-DD	Σ	Contains sufficient for < n > tests
	Consult Instruction for Use	2°C 8°C	Temperature Limitations
LOT	Batch Code		

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/literature/instructions-for-use-ifu/. Printed copies of an IFU document may be obtained by contacting Novodiax Technical Support or your local distributor.

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