NOVODIAX

Advancing immunoassays through innovation

ihcDirect® PRAME Ab-Enh
Anti-Human PRAME (Clone R1009)

Ab: K32042-015 and K32042-030, 150 and 300 tissue stains*
Ab K32042-005 and K32042-010 50 and 100 tissue stains*
Ab-Enh: K47042-015 and K47042-030, 150 and 300 tissue stains*
Ab-Enh: K47042-005 and K47042-010 50 and 100 tissue stains*

Intended Use: For *In Vitro* Diagnostic Use

Polymerized horseradish peroxidase (polyHRP)-labeled anti-PRAME (R1009) rabbit monoclonal antibody is intended for laboratory use to qualitatively identify by light microscopy the presence of PRAME protein in sections of formalin-fixed, paraffin-embedded (FFPE) and/or fresh 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 and proper controls interpreted by a qualified pathologist and/or physician. This conjugated antibody has been pre-diluted and optimized for IHC use without further dilution.

Summary and Explanation:

ihcDirect PRAME is a polymerized horseradish peroxidase (pHRP) conjugated rabbit monoclonal antibody (clone R1009). PReferentially expressed Antigen in Melanoma (PRAME) is encoded by the *PRAME* gene and has a molecular weight of approximately 57 kDa. PRAME is predominantly expressed in human melanomas and recognized by cytolytic T lymphocytes. The antibody may also have a role in acute leukemias and in preventing the growth or metastasis of breast cancer cells. A loss of PRAME may promote the growth and invasion of breast cancer cells.

Principle of Procedure:

The ready-to-use (RTU) ihcDirect PRAME pHRP antibody conjugate is directly applied to pretreated tissue sections, where it binds to PRAME antigens in tissue. Following antibody incubation, tissues are washed and a signal amplifier (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 PRAME 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
K47042-030* *K32042-030*	300*	2X15mL size ihcDirect PRAME RTU antibody conjugate and equal volume of ihc Enhancer. *2X15mL size ihcDirect PRAME RTU antibody conj.*
K47042-015* *K32042-015**	150*	15mL size ihcDirect PRAME RTU antibody conjugate and an equal volume of ihc Enhancer. *15mL size ihcDirect PRAME RTU antibody conjugate.*
K47042-010* *K32042-010**	100*	2X5mL size ihcDirect PRAME ready-to-use antibody conjugate and an equal volume of ihc Enhancer. *2X5mL size ihcDirect PRAME RTU antibody conjugate.*
K47042-005* *K32042-005**	50*	5mL size ihcDirect PRAME RTU antibody conjugate and an equal volume of ihc Enhancer. *5mL size ihcDirect PRAME RTU antibody conjugate.*

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

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Recombinant PRAME	R1009	Rabbit	IgG1	10 mg/mL

PRAME antibody is a rabbit monoclonal antibody purified from ascites. HRP is extracted from horseradish plant. The ihc Enhancer is a signal amplification reagent. Novodiax ihc DAB 1:1 Kit or DAB Kit are recommended for use with the PRAME antibody conjugate.

PRAME Ab-Enh Components (K47042-###):

Reagent Description	Component Part Numbers	Sizes (mL)
PRAME pHRP	H32042-R### (005, 015)	5, 15
ihc Enhancer	D28020-R### (005, 015)	5, 15

PRAME Ab Components (K32042-###):

Reagent Description	Component Part Numbers	Sizes (mL)
PRAME pHRP	H32042-R### (005, 015)	5, 15

Ancillary Reagents for Use with PRAME pHRP Antibody:

Reagent Description	Part Numbers	Sizes (mL)
ihc Enhancer	K51011-### (015, 030)	15, 30
ihc Blocker	K51001-### (015, 030)	15, 30
ihc Blocker (USA)	K51002-### (015, 030)	15, 30
ihc DAB 1:1 Kit	K50002-### (015, 030)	15, 30
DAB Kit	K50001-### (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 (Acetone and/or 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 (40-420x)

§ 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).
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).

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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 ihcDirect PRAME antibody conjugate and ihc Enhancer signal amplification reagent are pre-diluted. Further dilution may reduce signal intensity or increase the possibility of false-negative staining. 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: PRAME

1. Equilibrate all reagents to room temperature prior to use. Swirl or shake the ihc Enhancer and pHRP-labeled 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 (71.6°-86°F) for incubating PRAME pHRP and ihc Enhancer and a Novodiax chromogen WS. If room temperature is less than 21°C, users may need to incubate labeled 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 pHRP antibody, 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-minute IHC protocol for frozen tissue sections):

ihcDirect Antibody + Enhancer Frozen Tissue Procedure	Time in minutes
Fixation with NBF (10%)	Start
- ihc Wash (PBS-T), remove excess fluid	- - -
ihcDirect Ab	3
- ihc Wash (PBS-T), remove excess fluid	- - -
ihc Enhancer	3
- ihc Wash (PBS-T), remove excess	- - -
DAB (Working Solution)	1-3
- Wash (2x) ihc Wash or H_2O	- - -
Hematoxylin Counterstain/Rinse	2-30 Sec.
Rinse/Dehydrate/Mount media and coverslip	Varies by user
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 pHRP labeled anti-human PRAME antibody on slides covering the entire tissue and incubate for 30 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 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.
6. Counterstaining: Add hematoxylin and incubate for 1 minute at room temperature. Rinse twice with tap water for 2 minutes, each time.
7. 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.

8. 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 appropriately processed melanoma and skin. Nuclei are stained for melanoma cells in skin. One positive tissue control for each set of test conditions should be included in each staining run. Previously frozen, freshly cut tissues and in some cases, an individual's own tissue may be used as controls.

Tissues used for positive controls should be selected from patient specimens with characterized positive target activity that give positive staining results. Established positive tissue controls should only be utilized for monitoring correct performance of processed tissues and test reagents, rather than in formulating a specific diagnosis of patient samples. If positive tissue controls fail to demonstrate positive staining, patient specimen results should be considered invalid.

Negative Tissue Control: The same tissue may be used to provide positive and negative controls. Differentiation of cells present in most tissue sections provide internal negative control sites, but this should be verified by the user. Components that do not stain should lack antibody 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) pHRP-labeled 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 ihcDirect antibody incubation times by 1-2 minutes.
3. *High background*: Possible causes include (1) insufficient washing, (2) specimen drying out, (3) prolonged chromogen incubation, (4) prolonged pHRP-labeled antibody incubation, and (5) specimen 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 unexpected staining 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 color stains the tissue with a clean background if PRAME-expressed cells exist. There will be no color staining if no PRAME-expression cells exist in the tissue. 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 PRAME pHRP 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 sensitivedemonstrate high specificity in binding to the antigen of interest. Novodiax antibodies and accompanying reagents have demonstrated reliable and consistent results between runs and across lot numbers. 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 by testing materials at regular intervals and via surveillance programs.

ihcDirect PRAME Key to Symbols

IVD	*In vitro* diagnostic medical device	PRAME pHRP	pHRP PRAME antibody conjugate
REF	Catalog Number	ihc Enhancer	pHRP Enhancing amplification reagent
	Use by: YYYY-MM-DD	ihc Wash	ihc Wash Buffer
	Consult Instruction for Use		Manufacturer
LOT	Batch Code	2°C 8°C	Temperature Limitations
Σ	Contains sufficient for < n > tests		

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.

Bibliography:

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