Detection Combo - Standard Kit (Caliber30)

Laboratory use only

PRODUCT INFORMATION:

REF

PDC119 – 300 Test

INTENDED USE

For *laboratory use* only

Detection Combo – PDC119 is designed to be used with PathnSitu fully automated Slide Stainer Caliber30 and is intended for use in immunohistochemistry (IHC) staining procedures for the qualitative identification of antigens by light microscopy in formalin-fixed, paraffin-embedded (FFPE) tissues, cryostat tissues or cell preparations.

SUMMARY AND EXPLANATION

PathnSitu's IHC Detection Combo is a comprehensive solution designed for use with the Caliber30 Fully Automated Slide Stainer. The kit contains the key reagents and workflow accessories required for automated immunohistochemistry (IHC) staining, supporting tissue preparation, antigen retrieval, immunodetection, washing, and slide processing. The kit includes:

- Dewax Solution Pro – Used for automated deparaffinization of formalin-fixed paraffin-embedded (FFPE) tissue sections by effectively removing paraffin wax prior to staining.
- ER Solution I (High pH) – A Tris-EDTA based antigen retrieval solution (pH 9.0 ± 0.2) used to unmask epitopes in FFPE tissue sections, improving antigen accessibility for immunostaining.
- Caliber IM Wash Buffer (25X) – A concentrated wash buffer used throughout the staining process to remove excess reagents, maintain tissue integrity, and minimize non-specific background staining.
- PolyExcel HRP/DAB Detection 2 – A highly sensitive, non-biotin polymer-based detection system designed for use with mouse and rabbit primary antibodies. The HRP-labelled polymer technology enables sensitive antigen detection with low background staining.
- Positively Charged Slides – Specially treated slides that enhance tissue adhesion and help minimize tissue loss during staining and processing.
- Covershields – Protective covers used during automated staining to promote uniform reagent distribution, reduce reagent evaporation, and enable efficient staining with low reagent volumes, helping conserve reagents while maintaining consistent staining performance.
- Covershield Cleanser Tablets – Cleaning tablets designed for maintenance and effective reuse of Covershields, supporting reliable staining performance.

Together, these components provide a standardized and streamlined workflow for automated IHC staining on the Caliber30 platform, helping laboratories achieve consistent, reproducible, and reliable staining results while reducing manual intervention.

PRINCIPLE OF THE PROCEDURE

The IHC Detection Combo supports a fully automated immunohistochemistry workflow on the Caliber30 Automated Slide Stainer, including tissue deparaffinization, antigen retrieval, washing, target detection, chromogen development, and slide processing.

Following deparaffinization with Dewax Solution Pro, antigens in formalin-fixed paraffin-embedded (FFPE) tissue sections are unmasked using ER Solution I, a high-pH Tris-EDTA retrieval buffer. The tissue is then incubated with the appropriate primary antibody, followed by detection using the PolyExcel HRP/DAB Detection 2 system.

The PolyExcel HRP/DAB Detection 2 system employs a highly sensitive, non-biotin micro-polymer technology utilizing HRP-labeled polymers conjugated to secondary antibodies. The bound HRP catalyzes the conversion of DAB (3,3'-diaminobenzidine) substrate into an insoluble brown-colored precipitate at the site of antigen localization. Washing steps performed using Caliber IM Wash Buffer aid in the removal of unbound reagents and help minimize non-specific background staining. Following chromogen development, tissue sections are counterstained with hematoxylin to provide nuclear contrast and facilitate histological interpretation of the stained specimen.

KIT CONTENTS

Components	Cat#	Storage Condition	Pack Size
Caliber IM Wash Buffer (25X)	PAB117	2–8°C	250ml
Dewax Solution Pro	PAW119	RT	1000ml
ER Solution I (High pH)	PAR009	2–8°C	1000ml
PolyExcel HRP/DAB Detection 2	PAD116	2–8°C	300 Test
Covershields	PAS114	RT	30 Pieces
Covershield Cleanser	PAC111	RT	5 Tablets
Positively Charged Slides	PS011	RT	5 Boxes (72 Slides/Box)

MATERIALS REQUIRED BUT NOT SUPPLIED

- Control tissues (positive and negative controls)
- Isopropyl alcohol (IPA)
- Distilled or deionized (DI) water
- Coverslips or cover glass
- Mounting medium
- Primary antibody(ies)

STORAGE AND HANDLING

Storage Recommendations: Store at recommended temperatures. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use any component after the expiration date indicated on the product label.** If any reagent is stored under conditions other than those specified in this datasheet, its performance must be verified by the user prior to use and immediately place the vials at recommended temperatures away from sunlight in an upright position.

INSTRUCTIONS FOR USE

Dewax Solution Pro

1. Dewax Solution Pro is supplied ready-to-use and does not require dilution.
2. Pour the reagent into the bulk reagent bottle labeled "Dewax Solution" located in the Caliber30 Ancillary Station.

ER Solution I (High pH)

1. ER Solution I is supplied ready-to-use and does not require dilution.
2. Pour the reagent into the bulk reagent bottle labeled "ER Solution I" located in the Caliber30 Ancillary Station. The container can accommodate up to 2.5L.

Caliber IM Wash Buffer (25X)

1. Dilute the concentrated Caliber IM Wash Buffer with distilled or deionized water in a 1:25 ratio to prepare the working solution.
2. Pour the diluted wash buffer into the bulk reagent bottle labeled "Wash Buffer" located in the Caliber30 Ancillary Station. The container can accommodate up to 2.5 L.

PolyExcel HRP/DAB Detection 2

1. PolyExcel HRP/DAB Detection 2 is supplied in a barcoded reagent rack containing Caliber30 barcoded reagent vials.
2. Insert the reagent rack into the Reagent Station before each staining run.
3. Prior to first use, register the reagent rack barcode in the Reagent Management section of the Caliber30 software.
   Note: Follow the recommended staining protocol programmed within the Caliber30 Automated slide Stainer. Protocol parameters may be optimized based on laboratory requirements and specific assay conditions.

Covershield Cleanser:

1. Prepare the covershield cleanser solution by dissolving one of the supplied tablets in 1 L of distilled water.

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SPECIMEN PREPARATION

Staining Recommendations:
Routinely processed formalin-fixed, paraffin-embedded (FFPE) tissues and frozen tissue samples are suitable for use with PathnSitu's Detection Combo for Caliber30 and the appropriate primary antibody. The recommended fixative is 10% neutral buffered formalin. Tissue sections should be 2–5 µm thick.
Variable staining results may occur due to prolonged fixation, decalcification, or variations in tissue processing. Known positive and negative control tissues should be stained simultaneously with test specimens.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only.
2. This product is designed to use on Caliber30 Fully Automated Immunohistochemistry Automation only.
3. Proper handling of this product as with any product derived from biological sources should be used according to local and applicable regulations.
4. Do not use reagents after expiration date.
5. Use protective clothing and gloves, while handling reagents
6. All hazardous materials should be disposed according to local state and federal regulations.
7. Do not smoke, eat, or drink in areas where specimens or reagents are handled.
8. Any unused reagent should be stored according to the storage conditions specified for the respective component. Avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with copious amounts of water.

STAINING PROCEDURE

1. Prepare tissue sections on positively charged slides according to the laboratory's standard histology procedures.
2. Register slides with appropriate staining protocols in the Caliber30 software. Refer to the Caliber30 User Manual for detailed operating instructions.
3. Apply slide labels and load the slides into the appropriate slide trays. Place Covershields on the loaded slides to initiate the run.
4. Load the required primary antibodies and PolyExcel HRP/DAB Detection 2 reagents into the reagent station of the instrument.
5. Ensure that all required bulk reagents, including Dewax Solution Pro, ER Solution I, Caliber IM Wash Buffer and DI water, are adequately filled before starting the run.
6. Upon completion of the run, remove the slides from the instrument and place the Covershields in Covershield Cleanser for cleaning.
7. Dehydrate, clear, and coverslip the stained slides as per the laboratory's standard protocol.
8. For detailed instrument operation, protocol setup, and maintenance procedures, refer to the Caliber30 User Manual.

PROTOCOL NOTES

The Detection Combo is designed for use with PathnSitu's Caliber30 Fully Automated Slide Stainer, which is supplied with pre-programmed staining protocols. Users may optimize protocol parameters based on laboratory-specific requirements. The optimal antibody dilution and staining protocol may vary depending on factors including tissue fixation, incubation times, tissue section thickness, and antigen expression levels. The recommendations provided in this datasheet and the factory protocols of the Caliber30 are based on the exclusive use of PathnSitu products.

When using primary antibodies other than those recommended or validated by PathnSitu, it is strongly recommended to optimize staining conditions, particularly antibody dilution and incubation times, using appropriate positive and negative control tissues before testing clinical specimens.

Ultimately, it is the responsibility of the investigator to determine the optimal staining conditions for the intended application.

QUALITY CONTROL

PathnSitu follows and recommends to refer CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011. Always use positive and negative controls along with the test sample.

INTERPRETATION OF RESULTS

Stained slides should be evaluated by a qualified pathologist in conjunction with appropriate controls, tissue morphology, patient clinical history, and other relevant diagnostic findings. Results obtained using this kit should be interpreted within the context of the complete clinical picture and should not be used as the sole basis for diagnosis.

TROUBLESHOOTING

1. Follow the antibody-specific dilution and incubation recommendations provided by the manufacturer.
2. Staining results are dependent on proper tissue handling and processing. Improper fixation, tissue processing, antibody storage, washing, drying, heating, sectioning, or contamination may result in artifacts, antibody trapping, or inaccurate staining.
3. If protocol parameters are modified from the factory default settings, the optimized protocol should be verified using appropriate positive and negative control tissues before use on test specimens.
4. Ensure that Covershields are correctly positioned and are clean and free from discoloration prior to use.
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or e-mail: techsupport@pathnsitu.com.

TROUBLESHOOTING GUIDE

No Staining
Incorrect slide labeling or protocol assignment
Incorrect control tissue selected.
Primary antibody incubation time too short.
Covershield absent or incorrectly positioned

Weak Staining
Over-fixed or under-fixed tissue
Improper tissue processing
Primary antibody incubation time too short
Low antigen expression in the specimen

Non-specific or High Background Staining
Over-fixed or under-fixed tissue
Improper tissue processing
Excessive primary antibody concentration
Excessive antibody incubation time.
Necrotic tissue present in the specimen
Contaminated, discolored, or inadequately cleaned Covershields used

Tissues Lift Off
Non-charged or improperly charged slides used
Tissue section too thick
Improper tissue processing
Over-fixed or under-fixed tissue
Tissue containing excessive fat content
Inadequate slide drying or section adhesion prior to staining

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LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorized, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimize the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
11. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
12. PathnSitu makes no warranties beyond those expressly stated in the product description.
13. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
14. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
15. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS).

EXPLANATION OF SYMBOLS

LOT	**Lot number / Batch number**		**Expiry**
°C	**Storage limitation**		**Date of manufacture**
REF	**Catalogue number**		**Manufacturer address**
RT	**Room Temperature**		

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