Laboratory Use Only

PolyExcel HRP/DAB Detection System

(One Step Universal Kit for Mouse and Rabbit Primary Antibodies)

PRODUCT INFORMATION:

OSH001 6ml
OSH001 50ml
OSH001 100ml

INTENDED USE

For *laboratory* use only

PolyExcel detection system is intended to use with primary antibodies raised against mouse and rabbit for the qualitative identification of antigens by light microscopy in normal and pathological paraffin-embedded tissues, cryostat tissues or cell preparations.

SUMMARY AND EXPLANATION

PathnSitu's highly sensitive and specific PolyExcel detection system is non-biotin, polymer-based detection system which significantly reduce or shows no background on tissues containing high levels of avidin, biotin ex: Kidney, Liver and Lymphoid tissues. This system is based on an HRP labeled polymer, which is conjugated with secondary antibodies. The HRP solution is a single step solution, which is conjugated by Goat anti Mouse/Rabbit IgG.

PRINCIPLE OF THE PROCEDURE

Incubating the specimen for 5–10 minutes with peroxidase quencher, quenches any endogenous peroxidase activity. The specimen is then incubated with respective diluted mouse or rabbit primary antibody, followed by incubation with the PolyExcel HRP labeled polymer using recommended 15-20minutes incubation. Staining is completed by a 5–7 minutes incubation with 3,3'-diaminobenzidine (DAB) substrate-chromogen which results in a brown-colored precipitate at the antigen site (DAB is a potential carcinogen. Please take appropriate precautions).

KIT CONTENTS

PathnSitu PolyExcel detection kit supplied as in 3 different pack sizes. Details below:

Description	Cat#/Pack Size	Kit Contents
PolyExcel HRP/DAB Detection System	OSH001-6ml	PolyExcel Peroxidase Quencher
		PolyExcel Protein Block
	OSH001-50ml	PolyExcel Poly HRP
		PolyExcel Stunn DAB Substrate Buffer
	OSH001-100ml	PolyExcel Stunn DAB Substrate Chromogen

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Positive charged slides (PathnSitu Cat# PS011-72)
2. Control Tissues
3. Xylene
4. Isopropyl alcohol
5. DI Water
6. Hematoxylin
7. Cover glass
8. Mounting media
9. Antigen retrieval buffers (PathnSitu Cat# PS007, PS008, PS009)
10. Immuno wash Buffer (PathnSitu Cat# PS006)

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8^{o}C and away from light. Make sure to bring the solution to room temperature before use. Do not use after expiration date printed on the bottle. If reagents are stored under conditions other than those specified in the package insert, the user must verify them.

SPECIMEN PREPARATION

Staining Recommendations:

Routinely processed, FFPE tissues are suitable for use with desired primary antibody, when used PathnSitu's Poly Excel HRP/DAB detection system. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. Thickness of the sections should be 2-5µm. Slides should be stained once the sections are made as antigenicity of the cut sections may diminish over a period of time. It is recommended to stain known positive and negative controls simultaneously with unknown specimens.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. Proper handling of this product as with any product derived from biological sources should be used according to local and applicable regulations
3. Sodium azide inhibits peroxidase activity. Use caution when handling HRP conjugate to prevent any contamination with other reagents containing sodium azide
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. Avoid microbial contamination of reagents as it may lead to incorrect results

STAINING PROCEDURE

Preparation of working solutions: DAB (DAB is a potential carcinogen. Please take appropriate precautions): In a1ml of StunnDAB Buffer add 1drop of StunnDAB chromogen. Mix well the preparation and store it in dark. This solution is stable for a week when stored at 2-8^{o}C. Always prepare fresh for clean and crisp results.

Deparaffinization:

1. Deparaffinize tissue sections in 3 changes of xylene.
2. Hydrate slides in a series of graded alcohols to water.

Pretreatment Solution/Protocol: Please refer to the respective primary antibody datasheet for recommended pretreatment solution and protocol.

Staining protocol*:(* Wash tissue sections with immuno wash buffer after every incubation).

3. **Peroxidase Quencher:** Cover the tissue section with peroxidase quencher for 5-10 minutes.
4. **Protein Block (Optional):** Cover the tissue sections with Protein Block solution for 5-10 minutes. Strictly NO WASH, Tap the slides and proceed with next step.
5. **Primary Antibody:** Please refer to the respective primary antibody datasheet for incubation time and temperature.
6. **PolyExcel PolyHRP (Universal one step solution):** Cover the tissue sections with PolyExcel PolyHRP and incubate for 15-20 minutes at room temperature. Extended incubation may lead to back ground/Non specific staining.
7. **PolyExcel Stunn DAB working solution:** Cover the tissue sections with StunnDAB working solution (Please refer to preparation section on preparation of working solution) and incubate it for 5-7 minutes at room temperature.
8. **Hematoxylin:** Cover the tissue sections with Hematoxylin and incubate for appropriate time at room temperature.
9. Dehydrate slides through graded alcohols and xylenes then cover slip with appropriate mounting medium.

PROTOCOL NOTES

The optimum antibody dilution and protocols for a specific application can vary due to many factors. These include, but are not limited to: fixation, incubation times, and tissue section thickness and detection kit used. The data sheet's recommendations and protocols are based on exclusive use of PathnSitu products. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

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.

US Office:1257, Quarry Ln, Suite150, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

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ANALYTIC PERFORMANCE CHARACTERISTICS

1. Heat the paraffin-embedded tissue slides for a suitable duration at an appropriate temperature to promote tissue adhesion.

Note: Use positively charged coated slides (Cat no.: PS-011-72) for better adherence.

2. Deparaffinize the slides using xylene (preferably 3 changes with 5min each) to clear the paraffin wax present on and around the tissue.
3. Rehydrate the slides in graded alcohols (100%, 70%, and 50%) for 3 min each and in distilled water (preferably 2 changes with 2 min each) respectively.
4. Immerse the slides in 1X retrieval buffer (preferable Cat No.: PS009) and subject them to Heat-induced epitope retrieval by using a multi-epitope retrieval system (MERS-i) to unmask the epitopes.
5. Proceed further by using Poly Excel DAB Detection system (preferably Cat no: PEH002 or OSH001) kit components like Poly Excel Peroxidase Block to inactivate or block the non-specific binding firstly.
6. Apply the primary antibody specific to the target antigen. Incubate slides with the primary antibody for a suitable duration at an appropriate temperature as mentioned in the datasheet.
7. Rinse the slides to remove unbound primary antibody using wash buffer (preferably Cat no: PS006)
8. Apply the secondary antibody (preferably Poly Excel Poly HRP- Anti-Mouse/Anti-Rabbit Cat no: PEH002 or OSH001) conjugated to an enzyme that recognizes the primary antibody. Incubate slides with the secondary antibody for a suitable duration at an appropriate temperature.
9. Rinse the slides to remove unbound secondary antibodies using wash buffer (Preferably Immunowash buffer Cat no: PS006)
10. Apply a substrate, PolyExcel Stunn DAB Chromogen for enzyme-conjugated secondary antibody for a suitable duration.
11. Counter-stain the tissue section to visualize the expression in specific structures or cell types.
12. Dehydrate slides through graded alcohols (70%, 90%, 100%, 100%), clear the slides in Xylene (preferably 3 changes with 2min each) and mount the slides with an appropriate mounting medium.
13. Visualize the stained slides under the microscope.

The detection kit consistently exhibited specific and sensitive staining across various positive and negative tissue controls. This specificity and sensitivity were validated through inter-run, intra-run, and lot-based studies. The stability of the detection kit which was determined using real-time or accelerated methods extends until the expiration date indicated on the product labels.

TROUBLESHOOTING

1. Follow the antibody specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, antibody freezing and thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping or inaccurate results
3. Do not allow the section to dry out during the entire IHC process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu's Technical Support at +91-40-2701 5544 or E-mail:techsupport@pathnsitu.com

TROUBLESHOOTING GUIDE

No Staining:

1.	Critical reagent (such as primary or secondary antibody) omitted.
2.	Staining steps performed incorrectly or in the wrong order or sodium azide in buffer bath.
3.	Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4.	Wrong control identified.
5.	Primary antibody incubation period too short or secondary antibody at too low concentration.
6.	Improperly mixed substrate and/or chromogen solution(s).

Weak Staining:

1.	Tissue is either over-fixed or under-fixed.
2.	Primary antibody incubation time too short or Low expression of antigen.
3.	Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment
4.	Excessive rinsing during wash steps or incorrect procedure in reagent preparation.

Non-specific or High Background Staining:

1.	Tissue is either over-fixed or under-fixed or dried up during incubation period.
2.	Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised or requires longer incubation times.
3.	Highly concentrate reagents (Primary or secondary antibodies)
4.	Overly developed substrate.
5.	Tissue was inadequately rinsed or necrotic.

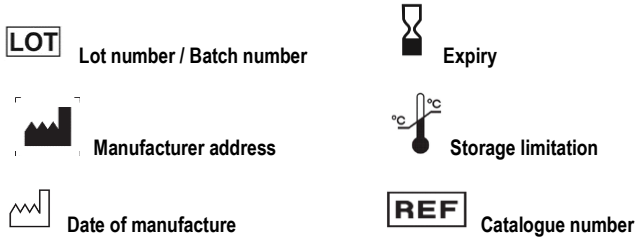
Tissues Lift Off

1.	Slides were not positively charged.
2.	Tissue was not baked properly.
3.	Tissue contained too much fat.

LIMITATIONS AND WARRANTY

1. Authorized and skilled/trained personnel only may use the product.
2. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results.
3. A qualified trained pathologist must perform the evaluation of the test results.
4. The product comes with no warranties beyond the provided description
5. Use appropriate volume/concentration to cover entire tissue sections and optimum conditions to avoid false positive and negative results.
6. Use appropriate/recommended buffer/instruments/all consumables with appropriate incubation timings to obtain optimal results.
7. Always recommend using known positive and negative controls to evaluate the test result.
8. Unexpected reactions may occur in untested tissues due to tissue component variability.
9. False positive results can arise from no stringent washing practices and other contributing factors.
10. In instances where localization differs from the specifications outlined in the datasheet, clinical coordination or prompt technical support is advised.
11. Maintain recommended storage conditions.
12. Refer entire data sheet to know any further limitations about the product.
13. No warranties whether expressed or implied, extend beyond the description.
14. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

EXPLANATION OF SYMBOLS



US Office:1257, Quarry Ln, Suite150, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
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Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com