

PolyExcel HRP/DAB Detection System Two Step Universal Kit for Mouse and Rabbit Primary Antibodies

PRODUCT INFORMATION:

PEH002	6ml
PEH002	50ml
PEH002	100ml

INTENDED USE

For *in vitro* diagnostic 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 two step detection system is non-biotin, micro-polymer-based detection system which significantly reduce or shows no back ground 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.

PRINCIPLE OF THE PROCEDURE

Incubating the specimen for 5–10 minutes with peroxidase quencher (H₂O₂) quenches any endogenous peroxidase activity. The specimen is then incubated with respective diluted mouse or rabbit primary antibody, followed by incubation with the PolyExcel Target Binder for 10 minutes then followed by a PolyExcel HRP labeled polymer using recommended 10minutes incubation. Staining is completed by 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 (Two step)	PEH002-6ml	PolyExcel Peroxidase Quencher (H ₂ O ₂) PolyExcel Target Binder
	PEH002-50ml	PolyExcel PolyHRP
	PEH002-100ml	PolyExcel Stunn DAB Substrate Buffer 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°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 a 1ml 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°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 (H₂O₂):** Cover the tissue section with peroxidase quencher for 5-10 minutes.
 4. **Primary Antibody:** Please refer to the respective primary antibody datasheet for incubation time and temperature.
 5. **PolyExcel Target Binder:** Cover the tissue sections with PolyExcel Target Binder and Incubate for 10minutes at room temperature.
 6. **PolyExcel PolyHRP:** Cover the tissue sections with PolyExcel PolyHRP and incubate for 10minutes at room temperature.
 7. **PolyExcel StunnDAB:** 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.

PERFORMANCE CHARACTERISTICS

The protocols for a specific application can vary. These include, but are not limited

to: fixation, heat-retrieval method, incubation times, and tissue section thickness and detection kit used. Due to the superior sensitivity of PathnSitu reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of PathnSitu products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

for property damage, personal injury, time or effort on economic loss caused by this product.

EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number



IVD *In vitro* diagnostic use



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

Authorized and skilled/trained personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified trained pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable