Stunn DAB Substrate Buffer & Chromogen Kit

IVD CE

PRODUCT INFORMATION:
PS001 100ml
PS001 60ml
PS001 10ml

INTENDED USE

For in vitro diagnostic use only
Stunn DAB Substrate Buffer & Chromogen Kit is intended for use in immunohistochemistry application. It is a high sensitivity DAB Chromogen suitable for use in combination with the PolyExcel HRP detection system or any other HRP based detection system. Upon oxidation, DAB forms a brown end-product at the site of the target antigen. The reagent is intended for use on formal-in-fixed, paraffin-embedded tissue sections.

SUMMARY AND EXPLANATION

Visualization is an important tool in the Immunohistochemistry process. The binding of primary and secondary antibodies in the process is illuminated by the enzymatic reaction of Horse radish peroxidase and chromogenic substrates such as DAB (3, 3'-Diaminobenzidine) and AEC (3-Amino-9-EthylCarbazole). The biochemical reaction converts the substrate into a pigment reaction product, which produces a colour such as Brown for DAB and Red for AEC. PathnSitu offers highly sensitive StunnDAB Chromogen and buffer for Immunohistochemical applications. It develops faster colour and has longer stability of working solutions up to 5 days when stored at 2-8°C. The chocolate/ golden brown colour develops during the reaction; it is very distinct and appealing under microscopic examination.

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored under the appropriate conditions, the antibody is stable until expiry. Do not use the product after the expiration date provided on the vial under any condition.
To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vial in an upright position in refrigerated conditions. The contents of the vial should be used within 9 months from the opening of the vial.

KIT CONTENTS

PathnSitu Stunn DAB Substrate Buffer & Chromogen Kit supplied in 3 different pack sizes. Details below:

Cat#	Pack Size	Kit components	Catalogue	Volume
PS001	10ml	Stunn DAB Substrate Buffer	IPS013	10ml
		Stunn DAB Substrate Chromogen	IPS014	1ml
	60ml	Stunn DAB Substrate Buffer	IPS013	60ml
		Stunn DAB Substrate Chromogen	IPS014	5ml
	100ml	Stunn DAB Substrate Buffer	IPS013	100ml
		Stunn DAB Substrate Chromogen	IPS014	4ml * 2

PREPARATION OF WORKING SOLUTION

For 1ml of working solution take 1ml of Stunn DAB Substrate Buffer add 1drop of Stunn DAB Substrate chromogen. Mix well the preparation and store it in dark. This solution is stable for a week when stored at 2-8ºC. Always prepare fresh working solutions for clean and crisp results.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only
2. The product is a potential carcinogen. Please take appropriate precautions, refer to the MSDS for further details
3. Do not use reagents after the expiration date
4. Use protective clothing and gloves while handling reagents
5. All hazardous materials should be disposed of according to local state and federal regulations
6. Avoid contamination of reagents as it may lead to incorrect results

STAINING PROCEDURE

After Polymer HRP incubation, wash slides with Immuno wash buffer, then cover the tissue sections with working solution of Stunn DAB Substrate buffer and Stunn DAB Substrate chromogen and incubate for 5-7 min at room temperature. Then wash with DI water, followed by Immuno wash buffer.

INTERPRETATION OF RESULTS

The diaminobenzidine-containing Substrate Working Solution gives a brown colour at the site of the target antigen recognized by the primary antibody. The brown colour should be present on the positive control specimen at the expected localization of the target antigen. If non-specific staining is present, this will be recognized as a rather diffuse, brown staining on the slides treated with the negative control reagent.

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 working solution of Stunn DAB Substrate buffer and Stunn DAB Substrate 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.

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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.
14. The Stunn DAB Substrate Buffer & Chromogen Kit consistently exhibited specific and sensitive staining across various positive and negative tissue controls with the respective primary antibody. This specificity and sensitivity were validated through inter-run, intra-run, and lot-based studies. The stability of the antibody, which was determined using real-time or accelerated methods, extends until the expiration date indicated on the product labels.

TROUBLESHOOTING

1. Follow the product specific protocol recommendations according to the 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

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. Use appropriate volume/concentration to cover entire tissue sections and optimum conditions to avoid false positive and negative results.
5. Use appropriate/recommended buffer/instruments/all consumables with appropriate incubation timings to obtain optimal results.
6. Always recommend using known positive and negative controls to evaluate the test result.
7. Unexpected reactions may occur in untested tissues due to tissue component variability.
8. False positive results can arise from no stringent washing practices and other contributing factors.
9. Maintain recommended storage conditions.
10. Please refer to the entire datasheet for additional instructions, precautions, and limitations.
11. No warranties whether expressed or implied, extend beyond description.
12. The kit has been validated on commonly tested formalin-fixed, paraffin-embedded (FFPE) tissues. Performance on other tissue types must be established by the user.
13. Do not use reagents that appear cloudy, discolored, or show signs of contamination. Discard if any component shows deterioration.
14. DAB is sensitive to light. Minimize exposure during handling and storage to preserve reactivity.
15. PathnSitu is not liable for property damage, personal injury, time or effort or economic loss caused by this product.

EXPLANATION OF SYMBOLS

Lot number / Batch number


Expiry

***In vitro* diagnostic use**

Storage limitation


Date of manufacture

Catalogue number


Manufacturer address


CE marking

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