Diagnostic BioSystems

IHC Made Affordable

SITVue/DAB Detection System

Catalog No: SIT-25D**,** SIT-100D, SIT-1000D
Document #: DS-6044-D
Effective Date: 12/18/2023

Intended Use: **For In Vitro Diagnostic Use**

SITVue /DAB Detection System is a powerful intensification system that significantly enhances chromogenic signals. This system can be integrated into standard immunohistochemistry (IHC) staining methods.

SITVue /DAB Detection System is an enzyme-mediated reaction that utilizes horseradish peroxidase (HRP) to catalyze the deposition of two separate Linkers applied sequentially onto tissue sections or cell preparation. The deposited Linkers can be detected with a streptavidin-peroxidase conjugate followed by a reaction with a peroxidase substrate/chromogen solution such as diaminobenzidine (DAB).

SITVue /DAB Detection System results in a significant increase in sensitivity compared to standard IHC detection methods, while maintaining similar specificity.

Principles of the Procedure: The principle of the procedure is that Linker 1 introduces HRP into the system. Linker 2 is to introduce the biotin into the system. Tracer introduces streptavidin ad additional HRP into the system. SITVue /DAB Detection System is an enzyme-mediated reaction that utilizes horseradish peroxidase (HRP) to catalyze the deposition of two separate Linkers applied sequentially onto tissue sections or cell preparation. The deposited Linkers can be detected with a streptavidin-peroxidase conjugate followed by a reaction with a peroxidase substrate/chromogen solution such as diaminobenzidine (DAB). SITVue /DAB Detection System results in a significant increase in sensitivity compared to standard IHC detection methods, while maintaining similar specificity.

Kit Contents

	25 Tests	100 Tests	1000 Tests
1. Tissue Primer	2.5 mL	10 mL	100 mL
2. Linker 1	2.5 mL	10 mL	100 mL
3. Linker 2	2.5 mL	10 mL	100 mL
4. Tracer	2.5 mL	10 mL	100 mL
5. Stable DAB/Plus Buffer	4 mL	15 mL	200 mL
6. Stable DAB/Plus Chromogen	0.5 mL	1 mL	5 mL
7. Empty mixing bottle for Stable DAB/Plus	15 mL bottle	15 mL bottle	15 mL bottle

Storage and Handling Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Stability 12-36 months (see expiration date on reagent bottles)

Composition All reagent components are formulated without azide or thimerosol preservatives. The reagents are provided in ready-to-use form, with the exception of Stable DAB/Plus.

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Material Required But Not Provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

Preparation of Stable DAB/Plus Substrate Working Solution

1. Transfer 1 mL of the Stable DAB/Plus Buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20µL) of Stable DAB/Plus Chromogen to the buffer. Mix thoroughly.
3. The substrate working solution is stable for 1 week refrigerated at 2-8°C.
4. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
5. Dispose of unused Stable DAB/Plus Substrate working solution in appropriate waste stream according to local, state, and federal regulations.

Precautions

This product is a single-use, non-sterile, in vitro diagnostic device.

i) DAB has been classified as a potential carcinogen and can cause skin irritation upon contact. Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
ii) Interpretation of the results is the sole responsibility of the user.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

Recommended Staining Protocol

1. Paraffin embedded tissue sections must be deparaffinized with xylene or dewaxing agent and rehydrated with a graded series of ethanol and water washes before staining. Follow the standard dewaxing and rehydration protocol used in your lab.
2. The investigator needs to optimize the dilution and incubation times for primary antibodies.
3. Each immunostaining run should include known positive and negative controls to assure proper functioning of the staining system and aid in valid interpretation of the results.

Typical controls:

Positive Control: A tissue known to contain the desired antigen, which has yielded positive staining in the past.

Negative Controls:
Reagent Controls

A. Substitute normal non-immune serum from the same host animal as the primary antibody (e.g. if using mouse monoclonal primary antibodies, use mouse non-immune serum).
B. Substitute matching host species isotype control for primary antibody
C. Use antigen-adsorbed primary antibody (i.e. antibody reagent which has been adsorbed with the target antigen to remove specific antibody)

Tissue control – A tissue known to not contain the desired antigen.

4. Consult the primary antibody supplier for recommended antigen recovery treatments. Perform epitope recovery pretreatments before starting the staining procedure.
5. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artifacts.

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STEP	STAINING PROCEDURE:	INCUBATION TIME
1. Tissue Primer	A. Apply the Tissue Primer and incubate. B. Rinse slides with 3 changes of Immuno Wash Buffer.	1 min. 3 x 10 second
2. Primary Antibody	A. Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration. Concentrated Primary Antibodies may be diluted using Primary Antibody Diluent. B. Wash slides with 3 changes of Immuno Wash Buffer.	5 min. 3 x 10 second
3. Linker 1	A. Apply the Linker 1 and incubate. B. Rinse slides with 3 changes of Immuno Wash Buffer.	5 min. 3 x 10 second
4. Linker 2	A. Incubate the tissue with Linker 2 . B. Wash slides with 3 changes of Immuno Wash Buffer.	5 min. 3 x 10 second
5. Tracer	A. Incubate the tissue with Tracer. B. Wash slides with 3 changes of Immuno Wash Buffer.	5 min. 3 x 10 second
6. Stable DAB/Plus	A. Prepare the Stable DAB/Plus substrate working solution (see above). B. Incubate tissue with prepared Stable DAB/Plus substrate solution. Monitor level of staining to determine optimal time of incubation. C. Rinse slides with 3 changes of water.	 5 min. 3 x 10 second
7. Counterstain	A. Incubate tissue with Counterstain (Hematoxylin K097), according to manufacturer's recommendation or standard laboratory protocol. B. Wash slides with water 3 times, followed by 1 time in Immuno Wash Buffer, then 1 time in water.	~1 min. 3 x 10 second H2O 1 x 10 second Buffer 1 x 10second H2O
8. Dehydrate & Coverslip	A. Dehydrate tissues through graded ethanol series, followed by xylene series. B. Apply coverslips with permanent mounting medium.	

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