BrightDAB, substrate DAB
(For use with HRP-labeled detection systems)

Instruction For Use

These instructions apply to the WellMed BrightDAB substrate.

1: Intended Use

For in-vitro Diagnostic Use.
WellMed BrightDAB substrate DAB is intended for use in immunohistochemistry.

2: Summary and explanation

WellMed BrightDAB substrate DAB (3,3'Diaminobenzidine) is a widely used chromogen for immunohistochemical staining and immunoblotting. When in the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol and xylene. This product comes in a two-component system consisting of a liquid stable DAB chromogen and DAB substrate buffer. DAB Solution A: Ready–to-Use Buffered H_2O_2,; DAB Solution B: Concentrated DAB solution. BrightDAB is a very stable and superior formulation of DAB. In some cases, antibodies titers may increase by two-fold. BrightDAB can be used both manually and on automated stainers.
The clinical interpretation of any staining or its absence should be determined by a qualified pathologist and complemented by morphologic studies; controls should be evaluated within the context of the patient's clinical history and/or other diagnostic tests.

3: Kit components

BrightDAB, substrate DAB; consists of two components:
1. DAB Solution A: Buffered H_2O_2 (Ready-to-Use)
2. DAB Solution B: Concentrated DAB solution

4: Availability

Catalog Number	Contents	Volume
BS04-110	BrightDAB, substrate DAB	
	• Sub cat.no.: BS04-110A: DAB Solution A: Buffered H_2O_2 (Ready-to-Use)	110 ml
	• Sub cat.no.: BS04-110B: DAB Solution B: Concentrated DAB solution	5 ml

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BS04-500	BrightDAB, substrate DAB • Sub cat.no.: BS04-500A: DAB Solution A: Buffered H_2O_2 (Ready-to-Use) • Sub cat.no.: BS04-500: DAB Solution B: Concentrated DAB solution	 500 ml 22 ml
BS04-999	BrightDAB, substrate DAB • Sub cat.no.: BS04-999A: DAB Solution A: Buffered H_2O_2 (Ready-to-Use) • Sub cat.no.: BS04-999B: DAB Solution B: Concentrated DAB solution	 1000 ml 45 ml

5: Usage

1 Working solution: Add 40 µl DAB Solution B (± one drop) to 1 ml Solution A, mix well.
2 Incubate the DAB solution one time 8 minutes without washing in between.

6: Recommended Staining Protocol

Step	Reagent	Template step	Incubation time
1	Deparaffinize and rehydrate tissue section	Slide/tissue preparing	-
2	Wash aqua dest	Wash	2x 5 min
3	If applicable; HIER or digestive enzyme	Pre-treatment	*
4	Wash buffer	PBS or TBS buffer	2x 5 min
5	H_2O_2 (concentration 3%)	Tissue preparing	10 min
6	Wash buffer	PBS or TBS buffer	2x 5 min
7	Primary antibody	Antibody	30 min
8	Wash buffer	PBS or TBS buffer	2x 5 min
9	Detection system, polymer HRP	Labeled polymer	30 min
10	Wash buffer	PBS or TBS buffer	2x 5 min
11	***Substrate***	DAB	8 min
12	Wash aqua dest	Wash	2x 2 min
13	Counterstain, dehydrate and coverslip	Auxiliary	-

* See applicable IFU

7: Control slides

A positive control, negative control and reagent control are needed and processed in the same way as the unknow specimen slide to interpret staining results.

8: Storage

Store at 2-8 °C and in the dark. Do not use after expiration date.

9: Warnings and precautions

Refer to SDS.

10: Troubleshooting

Please contact WellMed by phone or by email.

11: Reference

1) Shan-Rong Shi, James Guo, Richard J. cote, Lillian Young, Debra Hawes, Yan Shi, Sandra Thu and Clive R. Taylor, Applied Immunohistochemistry & Molecular Morphology, vol 7,201-208,1999