INSTRUCTIONS FOR USE

Chromogenic substrate kit (with ready-to-use and concentrated components)

CE IVD

DAB Substrate Kit

REF	Description
DAB-057	1 Kit, 500 Tests
DAB-530	1 Kit, 5,000 Tests

1 Specifications

Chromogenic enzyme substrate for visualization of an antibody-enzyme conjugate-linkage during IHC on human FFPE tissue sections.

2 Intended purpose

The DAB Substrate Kit is used for staining procedures in qualitative immunohistochemistry (IHC) in combination with a horseradish peroxidase (HRP) based detection system and for the qualitative detection of antigens (IHC) in human formalin-fixed, paraffin-embedded (FFPE) tissue sections. DAB (3,3'-diaminobenzidine) forms a permanent brown precipitate through oxidation at the location of the target antigen, which is insoluble in aqueous or organic solvents and can be visualized with the light microscope. The product is intended for professional laboratory use by qualified personnel. The DAB Substrate Kit has been tested for use in manual and automated procedures. The product is an accessory to an *in-vitro* diagnostic medical device and intended to be used in combination with reagents and solutions from Zytomed Systems necessary for immunohistological staining (e.g. primary antibody). The accessory supports the detection of a physiological or pathological state by the *in-vitro* diagnostic medical device (e.g. primary antibody).

3 Test principle

Immunohistochemistry (IHC) is a method that combines histological and immunological techniques. A primary antibody is used for the detection of a specific antigen. The detection of the antigen is based on the affinity of the antibody for this antigen, which leads to a specific bond between the two. The combination with an enzyme-linked detection system enables the visualization of the antigen by the successive use of the specific primary antibody against the antigen, a secondary antibody or linker against the primary antibody, an enzyme conjugate and a chromogenic substrate in combination with intermediate washing steps. The enzymatic activation of the chromogen leads to a visible product at the antigen site in the tissue. The tissue section is counterstained, sealed with a coverslip and the result is interpreted under the light microscope.

4 Reagents provided

The product is provided in the following formats with additives for preservation and stabilization.

REF	Description	Composition
DAB-057	DAB Chromogen, 3 ml concentrate; DAB Substrate Buffer, 11 x 5 ml ready-to-use	Liquid DAB concentrate, Substrate buffer with hydrogen peroxide
DAB-530	DAB Chromogen, 30 ml concentrate; DAB Substrate Buffer, 500 ml ready-to-use	Liquid DAB concentrate, Substrate buffer with hydrogen peroxide

A safety data sheet can be requested under info@zytomed-systems.de and is available under www.zytomed-systems.de.

5 Materials required but not provided

- Pretreatment buffer
- Primary antibody
- Antibody diluent (for concentrated antibodies only)
- Wash buffer
- Deionized or distilled water
- Xylene or xylene substitute
- Ethanol or 2-propanol
- Where appropriate avidin-/biotin-blocking solution
- Where appropriate peroxide-blocking solution
- Detection system
- Hematoxylin or another counter staining
- Mounting medium
- Where appropriate steamer or water bath
- Where appropriate automated staining system
- FFPE tissue sample
- Positive and negative control specimens
- Adhesive slides
- Coverslips
- Staining vessels/tanks
- Thermometer
- Timer
- Microscope

6 Storage and handling

The stability of this product was verified according to EN ISO 23640.
Store at 2-8 °C. Do not freeze the product. Return to storage conditions immediately after use. Store the chromogen (concentrate) protected from light. Avoid microbiological contamination of the product. Open the container only to remove a part of the product and then close it immediately. The product is stable until expiry date indicated on the label when handled accordingly. Do not use the product beyond expiry date indicated on the label. Staining solutions prepared from concentrates shall be used immediately.

7 Specimen collection and preparation for analysis

- Fix the human tissue sample and the tissue control in 4% neutral buffered formaldehyde (10% neutral buffered formalin solution, respectively).
- Embed the fixed tissue samples in paraffin.
- Make tissue sections with a microtome. The recommended slice thickness is 2-4 µm.
- Apply the tissue sections without wrinkles to adhesive slides and label them according to your internal standards.

8 Staining procedure

The product is intended for use in combination with other reagents. Please note that the chromogenic substrate is only suitable for use in combination with a horseradish peroxidase (HRP) based detection system. Zytomed Systems GmbH validated the use of the product in combination with the following reagents and devices:

- All CE/IVD-labelled primary antibodies from Zytomed Systems
- If applicable, CE/IVD-labelled dilution buffer from Zytomed Systems matching the primary antibody
- If applicable, CE/IVD-labelled pre-treatment buffer from Zytomed Systems matching the primary antibody
- CE/IVD-labelled wash buffer from Zytomed Systems
- CE/IVD-labelled secondary antibody or polymer from Zytomed Systems matching the primary antibody
- Immunostainer IntelliPathFLX® from BioCare Medical

It is possible to use the product with deviant reagents, devices, and protocols that meet equivalent performance indicators. In this case, the user is responsible for validating

the chromogenic substrate, the test system, and the protocol used in the respective clinical context.

Manual and automated procedure (IntelliPathFLX® device from BioCare Medical)		
Parameter	**Zytomed Systems recommendations**	
	REF	**Dilution**
Preparation of staining solution	DAB-057	Add 4 drops (200 µl) of DAB Chromogen to one bottle of DAB Substrate Buffer, mix thoroughly.
	DAB-530	Add 50 µl DAB Chromogen to 1 ml DAB Substrate Buffer, mix thoroughly.
Incubation time	• ~ 10 min, controlling the colour intensity via light microscope is recommended	
Staining	• Wash slide with CE/IVD-labelled wash buffer from Zytomed Systems two times after previous incubation step. • Apply DAB chromogen working solution to the slide. • Wash slide with CE/IVD-labelled wash buffer from Zytomed Systems two times	
Counterstaining	• Counterstain with haematoxylin for 30 sec to 10 min at room temperature (*depending on the desired coloring intensity*) • Rinse slide with running tap water for ~ 3 min.	
Dehydration	• Dehydrate in an ascending alcohol series and permanently cover from xylene	
Mounting	• Permanent mounting	

9 Quality control

We recommend using a positive and a negative control with every staining run. The positive control is used to check the correct processing of the sample. If the negative control is positive, this indicates an unspecific staining.

10 Expected results

The expected results depend on the use of the primary antibody, the detection system, the appropriate counterstain as well as the suitable mounting medium. By using a suitable chromogenic substrate which forms a colored precipitate at the site of antigen-antibody binding, the linkage of antibody and enzyme conjugate can be durably visualized.

11 Analytical performance characteristics

Analytical performance studies for the parameter precision (including repeatability and reproducibility) and endogenous interferences were performed. The relevant experiments met the pre-specified acceptance criteria of at least 90 % overall percent agreement for all parameter tested. For potential endogenous interferences please refer to section limitations in the instruction for use. Hence, DAB (DAB Substrate Kit) achieves analytical performance as required by Regulation (EU) 2017/746, Annex I, 9.1(a), when used properly and taking into consideration the generally acknowledged state of the art.

12 Troubleshooting

If you observe unusual staining or other deviations from the expected results, please read these instructions carefully. Refer to the instructions of the detection system for further relevant information. Our experts are available to answer your questions. Please contact info@zytomed-systems.de.

13 Limitations

- For *in-vitro* diagnostic use.
- For professional use only. Staining must be performed in a professional laboratory by qualified personnel under the supervision of a pathologist/clinician who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
- The clinical interpretation of any positive staining, or its absence, must be done within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. It is the responsibility of a qualified pathologist/clinician to be familiar with the product, accessory reagents, diagnostic panels, and methods used to produce the stained tissue.
- Specimen staining, especially signal intensity and background staining, is dependent on the handling and processing of the specimen prior to staining. Incorrect tissue processing or inappropriate handling of the tissue samples before the actual IHC staining can lead to inaccurate results.
- The endogenous peroxidase activity, the pseudo peroxidase activity in erythrocytes or the endogenous biotin content can cause unspecific staining depending on the detection system used.
- Inadequate counterstaining or incorrect mounting can affect the interpretation of the results.
- Zytomed Systems GmbH guarantees that the product, if stored and handled correctly, meets all the requirements described up to the expiry date stated on the product label. No further guarantees can be given.
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user. This CE/IVD is compliant to Regulation (EU) 2017/746 only if used as described in these instructions for use within the scope of the intended purpose.

14 Important user information

- The product is to be used as an accessory of an *in vitro* diagnostic medical device in professional application. It supports the detection of a pathological or physiological condition by this *in vitro* diagnostic medical device.
- Serious incidents that occur in connection with the product must be reported to the manufacturer and the competent authority of the Member State in which the user is located.

15 Warnings and precautions

- Read the safety data sheet before using the product.
- Do not use the product if it is damaged.
- Do not use the product if you notice anything unusual about the product that indicates contamination, such as an unusual cloudiness or odor. In this case, please contact our customer service.
- Wear protective equipment to avoid eye, skin, or mucosal contact with the reagent. If you come into contact with the reagent, wash it with plenty of water.
- Avoid microbiological contamination of the product, otherwise an unspecific colouring could occur. Open the container only to remove a part of the product and then close it immediately. Store the product at the recommended storage temperatures.

- Open the required reagent only for the withdrawal of partial quantities and carefully label any secondary containers used to minimize the risk of confusion in the case of solutions of the same color.
- For DAB-057, note the level of DAB Substrate Buffer when adding the DAB Chromogen (4 drops of DAB Chromogen to 1 bottle of DAB Substrate Buffer) to achieve the correct concentration of ready-to-use solution.
- When handling substances that are considered CMR substances (e.g. xylene), ensure that the technical and personal protective equipment is adapted to the substance.
- Dispose of the product according to the information in the safety data sheet and in accordance with regional regulations. The chromogen (-concentrate) is disposed of via hazardous waste.
- Samples of human origin and therefore contaminated consumables must be disposed of in accordance with regional legal regulations.

16 Literature

1. Elias JM “Immunohistopathology – A practical Approach to Diagnosis” ASCP Press 2003
2. Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

www.zytomed-systems.de

Zytomed Systems GmbH • Anhaltinerstraße 16 • 14163 Berlin, Germany • Tel: (+49) 30-804 984 990

17 Explanations of the symbols on the product label

Symbols are used in accordance with ISO 15223-1. Further symbols on the product label might be:

GSH02: Flammable

GSH05: Caustic

GSH07: Attention / Warning

GSH08: Systemic health hazards

RUO For Research Use Only

18 Changes compared to the previous version

Version	Changes
02	• Adaptation to current template V05 • Addition of notes in section 15 • General editorial corrections