

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

DAB High Contrast Kit

REF	DABPLUS-500	1 Kit, 500 tests
	DABPLUS-5000	1 Kit, 5000 tests

DABPLUS-5000	DAB Chromogen, 30 ml concentrate; DAB Substrate Buffer High Contrast, 500 ml ready-to-use	Liquid DAB concentrate, Substrate buffer High Contrast with hydrogen peroxide
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For use in qualitative immunohistochemistry (IHC)

In vitro diagnostic medical device according to IVDR (EU) 2017/746

1. Specifications

Chromogenic enzyme substrate for visualization of antibody-enzyme conjugate binding in the context of IHC on human FFPE tissue sections.

2. Intended purpose

The DAB High Contrast Kit is used for staining procedures in 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 DAB High Contrast Kit is particularly suitable for applications in which a particularly clear contrast between the chromogen and the counterstaining with hemalum or hematoxylin is desired. The colour reaction results in a more intense brown shade compared to the DAB Substrate Kit. The product is intended for professional laboratory use by qualified personnel. The DAB High Contrast 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 GmbH and ZytoVision GmbH 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 stabilisation.

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

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

5. Materials required but not provided

- Pretreatment buffer
- Dilution buffer (only for concentrated antibody)
- Primary antibody
- 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, steam pressure pot or water bath
- Where appropriate staining automat
- FFPE tissue sample
- Positive and negative control specimens
- Adhesive slides
- Coverslips
- Staining vessels/tanks
- Thermometer
- Timer
- Microscope

6. Preparation of specimens

- 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 internal standards.

7. Assay procedure

The product is intended for use in combination with other reagents. Zytomed Systems GmbH validated the use of the product in combination with the following reagents and devices:

- All primary antibodies (CE/IVD) of ZytoVision GmbH and Zytomed Systems GmbH
- Where appropriate dilution buffer (CE/IVD) of ZytoVision GmbH
- No or heat pretreatment with a pretreatment buffer (CE/IVD) of ZytoVision GmbH
- Wash buffer (CE/IVD) of ZytoVision GmbH;
 - Recommendation for manual IHC: ZUC052
 - Recommendation for automated IHC using IntelliPathFLX® of BioCare Medical: ZUC066
- Polymer/Secondary antibody (CE/IVD) of ZytoVision GmbH
- Detection system (CE/IVD) of ZytoVision GmbH
- Automated IHC: IntelliPathFLX® of BioCare Medical

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

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user is responsible for validating the antibody, the test system, and the protocol used in the respective clinical context.

Please follow the recommendations below for the staining procedure. Please also take into account the information about the staining protocol in the instructions for use of the detection system you are using.

Manual and automated procedure (IntelliPathFLX® device from BioCare Medical)		
Parameter	Zytomed Systems recommendations	
	REF	Dilution
Preparation of staining solution	DABPLUS-500	Add 5 drops of DAB Chromogen to one bottle of DAB Substrate Buffer High Contrast, mix thoroughly
	DABPLUS-5000	Add 50 µl DAB Chromogen to 1 ml DAB Substrate Buffer High Contrast, mix thoroughly
Incubation time	• ~ 10 min, controlling the colour intensity via light microscope is recommended	
Staining	<ul style="list-style-type: none"> • Wash slide with CE/IVD-labelled wash buffer from Zytomed Systems two times after previous incubation step • Apply DABPLUS chromogen working solution to the slide • Wash slide with CE/IVD-labelled wash buffer from Zytomed Systems two times 	
Counterstaining	<ul style="list-style-type: none"> • Counterstain with haematoxylin for 30 sec to 10 min at room temperature (<i>depending on the desired coloring intensity</i>) • Rinse slide with running tap water for ~ 3 min 	
Dehydration	<ul style="list-style-type: none"> • Dehydrate in an ascending alcohol series and permanently cover from xylene 	
Mounting	<ul style="list-style-type: none"> • Permanent mounting 	

8. Storage and handling

The stability of this product was verified according to DIN EN ISO 23640. Store at 2-8 °C. Do not freeze the product. Return to storage conditions immediately after use. 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. For concentrated antibodies, the stability of the working solution must be validated by the user.

9. Warnings and precautions

- Read the safety data sheet before using the product.
- Do not use the product if it is damaged, if you observe an unexpected colour change in the product or unexpected turbidity occurs.
- Mix the product well before use.
- When staining, ensure that the reagents used are compatible and that the staining is done at room temperature.
- The product must be validated by the user before use for diagnostic purposes outside the intended purpose or in the context of an LDT application.
- 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 in order to minimise the risk of confusion in the case of solutions of the same colour.
- 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.
- Samples of human origin and therefore contaminated consumables must be disposed of in accordance with regional legal regulations.
- 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.

Hazard and precautionary statements for ZUC017:

The hazard-determining components are hydrochloric acid, biphenyl-3,3',4,4'-tetrayltetraamine; diaminobenzidine.

		Danger
H314		Causes severe skin burns and eye damage.
H341		Suspected of causing genetic defects.
H350		May cause cancer.
P201		Obtain special instructions before use.
P260		Do not breathe dust/fume/gas/mist/vapours/spray.
P280		Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.
P303+P361+P353		IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305+P351+P338		IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310		Immediately call a POISON CENTER/doctor.

10. Limitations

- For *in-vitro* diagnostic use.
- For professional use only. Staining must be performed in a professional laboratory by qualified personnel with suitable, calibrated laboratory equipment 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 as well as the reagents prior to staining. Incorrect tissue processing, inappropriate handling of the tissue samples or incorrect preparation or dilution of reagents before the actual IHC staining can lead to inaccurate results. When handling several types of tissues or reagents at the same time, always ensure correct processing to avoid confusion.
- 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.

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- Inadequate counterstaining or incorrect mounting can affect the interpretation of the results.
- ZytoVision 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 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.

11. Interfering substances

Endogenous peroxidase activities can cause non-specific staining when using HRP-based detection systems. This can be minimized by inactivating endogenous peroxidases using H₂O₂ or a peroxide block. Endogenous biotin can cause non-specific staining when using avidin-biotin based detection systems. This can be minimized by adequate protein blocking. This is already included in dilution buffers of ZytoVision GmbH as well as in ready-to-use primary antibodies of ZytoVision GmbH and Zytomed Systems GmbH.

12. Interpretation of results

The interpretation of the results is the responsibility of the professional user.

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

13. Recommended quality control procedures

We recommend carrying out 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. For suitable positive and negative controls please refer to the instruction for use of the primary antibody.

14. Performance characteristics

Analytical performance studies were performed for precision.

The following precision analysis were performed:

- Intra-day precision (repeatability)
- Inter-day precision (reproducibility)
- Lot-to-lot precision
- Inter-platform precision between different stainers of the same manufacturer (IntelliPathFLX® of BioCare Medical)

The predefined acceptance criteria for all tested parameters were fulfilled. Thus, the device achieves the analytical performance required by Regulation (EU) 2017/746, Annex I, 9.1(a), when used as intended and taking into account the generally acknowledged state of the art.

Clinical performance testing is not required as the device is categorized as risk class A and does not detect an analyte itself but is used as an accessory in an *in-vitro* diagnostic procedure.

15. Disposal

The disposal of reagents must be carried out in accordance with local regulations.

16. Troubleshooting

Any deviation from the operating instructions can lead to inferior staining results or to no staining at all. Our experts are available to answer your questions. Please contact info@zytomed-systems.de.

17. 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

Additional relevant literature was identified during the systematic literature review on SoA and scientific validity.

18. Revision



www.zytomed-systems.de

Please refer to www.zytomed-systems.de for the most recent instructions for use.



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