CE IVD

Detection system (kit with ready-to-use and concentrated components)

ZytoChem Plus (HRP) Broad Spectrum (DAB) Kit

REF HRP008DAB Σ 1 Kit, 80 tests

For use in qualitative immunohistochemistry (IHC)

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

1. Specifications

Detection system in the context of IHC for visualization of a target antigen in human FFPE tissue.

2. Intended purpose

The ZytoChem Plus (HRP) Broad Spectrum (DAB) Kit works according to the streptavidin-biotin principle and is used for the qualitative detection of antigens during immunohistochemistry (IHC) on human formalin-fixed, paraffin-embedded (FFPE) tissue sections. The kit contains a polyvalent biotinylated secondary antibody that binds mono- and polyclonal primary antibodies from mouse and rabbit. The product is intended for professional laboratory use by qualified personnel. The ZytoChem Plus (HRP) Broad Spectrum (DAB) 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
HRP008DAB	• Peroxide Block 8 ml, ready-to-use • Blocking Solution (1) 8 ml, ready-to-use • Biotinylated Secondary Antibody polyvalent (2) 8 ml, ready-to-use • Streptavidin-HRP Conjugate (3) 8 ml, ready-to-use • DAB Substrate Buffer 7 x 5 ml, ready-to-use • DAB Chromogen 3 ml concentrate BIO	Detection kit with Peroxide Block, Blocking solution containing casein protein, biotinylated goat secondary antibody molecules, streptavidin molecules conjugated to horseradish peroxidase (HRP) in buffer supplemented with a mixture of isothiazolones for stabilization, DAB Substrate Buffer, and DAB Chromogen concentrate containing <4% hydrogen chloride and <2% diaminobenzidine

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

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Please follow the recommendations below for the staining procedure.

Manual procedure *Note: A wash step must be performed after each reagent: 2x with CE/IVD-labeled wash buffer from Zytomed Systems (e.g. ZUC052).*		
Parameter	**Zytomed Systems recommendation**	
	REF	**Dilution**
Preparation of staining solution	DAB Chromogen	4 drops / 200 µl DAB chromogen concentrate for 1 bottle (5 ml) DAB substrate buffer
	DAB Substrate Buffer	5 ml DAB substrate buffer
Peroxide Block	• 10 min	
Blocking Solution (protein blocking)	• **Reagent 1** • 5-10 min at room temperature (*This step is optional.*)	
Primary antibody (or negative control reagent)	• Information on the dilution of antibody concentrates as well as incubation times and temperatures for primary antibodies can be found in the product-specific instructions for use	
Biotinylated Secondary Antibody, polyvalent	• **Reagent 2** • 10-15 min at room temperature	
Streptavidin-HRP-Conjugate	• **Reagent 3** • 10-15 min at room temperature	
Chromogenic substrate	• **DAB working solution** • 5-15 min (check color development under the light microscope)	
Stopping the reaction	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)	
Counterstaining	• Hematoxylin for 30 sec to 10 min (depending on the desired staining intensity) at room temperature. • Bluish in warm tap water for ~3 min	
Dehydration	• Dehydrate in the ascending alcohol series and permanently cover from the xylene	
Mounting	• permanent	

Automated procedure in the IntelliPathFLX® device from BioCare Medical *Note: A wash step must be performed after each reagent: 2x with CE/IVD-labeled wash buffer from Zytomed Systems (e.g. ZUC066).*		
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	REF	**Dilution**
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Streptavidin-HRP-Conjugate	• **Reagent 3** • 10-15 min at room temperature	
Chromogenic substrate	• **DAB working solution** • 5-15 min (depending on the desired staining intensity)	
Stopping the reaction	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC066)	

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.
- The product contains material of animal origin.
- 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.
- For stabilisation, a mixture of isothiazolones / stabilisers of isothiazolones are used. Disposal is via hazardous waste.
- 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.

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Hazard and precautionary statements for ZUC012:

The hazard-determining component is a reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	IF skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

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.
- 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 H_2O_2 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 and analytical specificity.

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 evaluation of analytical specificity was performed using primary antibodies originated from mouse and rabbit.

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.

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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. Omata M et al. Am J Clin Pathol 73: 626-632, 1980
3. Nadji M and Morales AR. Ann N.Y. Acad Sci 420:134-139, 19834

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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