INSTRUCTIONS FOR USE

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

CE IVD

ZytoChem Plus (HRP) Broad Spectrum Kit

REF	Description
HRP-060	1 Kit, 600 Tests
HRP-125	1 Kit, 1,250 Tests
HRP-500	1 Kit, 5,000 Tests
HRP-500X	1 Kit without Blocking Solution, 5,000 Tests

1 Specifications

Detection system within IHC staining for the visualization of a target antigen on human FFPE tissue sections.

2 Intended purpose

The ZytoChem Plus (HRP) Broad Spectrum 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. It must be used in combination with substrates compatible with the enzyme horseradish peroxidase (HRP). The product is intended for professional laboratory use by qualified personnel. The ZytoChem Plus (HRP) Broad Spectrum 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
HRP-060	• Blocking Solution (1) 15 ml, ready-to-use • Biotinylated Secondary Antibody polyvalent (2) 15 ml, ready-to-use • Streptavidin-HRP Conjugate (3) 15 ml, ready-to-use BIO	Detection kit with Blocking solution containing casein protein, biotinylated goat secondary antibody molecules and streptavidin molecules conjugated to horseradish peroxidase (HRP) in buffer supplemented with a mixture of isothiazolones for stabilization
HRP-125	• Blocking Solution (1) 125 ml, ready-to-use • Biotinylated Secondary Antibody polyvalent (2) 125 ml, ready-to-use • Streptavidin-HRP Conjugate (3) 125 ml, ready-to-use BIO	Detection kit with Blocking solution containing casein protein, biotinylated goat secondary antibody molecules and streptavidin molecules conjugated to horseradish peroxidase (HRP) in buffer supplemented with a mixture of isothiazolones for stabilization
HRP-500	• Blocking Solution (1) 500 ml, ready-to-use • Biotinylated Secondary Antibody polyvalent (2) 500 ml, ready-to-use • Streptavidin-HRP Conjugate (3) 500 ml, ready-to-use BIO	Detection kit with Blocking solution containing casein protein, biotinylated goat secondary antibody molecules and streptavidin molecules conjugated to horseradish peroxidase (HRP) in buffer supplemented with a mixture of isothiazolones for stabilization
HRP-500X	• Biotinylated Secondary Antibody polyvalent (2) 500 ml, ready-to-use • Streptavidin-HRP Conjugate (3) 500 ml, ready-to-use BIO	Detection kit with biotinylated goat secondary antibody molecules and streptavidin molecules conjugated to horseradish peroxidase (HRP) in buffer supplemented with a mixture of isothiazolones for stabilization

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
- Chromogenic substrate (only necessary if not included in the detection kit used)
- 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. 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.

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 internal standards.

8 Staining procedure

The product is intended for use in combination with other reagents. Please note that an additional chromogenic substrate, suitable for the combination with the detection system horseradish peroxidase (HRP), is necessary. 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 chromogenic substrate from Zytomed Systems matching the secondary antibody/polymer
- 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 detection kit, the test system, and the protocol used in the respective clinical context.
Please follow the recommendations below for the staining procedure.

Manual procedure	
Parameter	**Zytomed Systems recommendation**
Peroxide blocking	• 3% H_2O_2 solution • 10 min at room temperature
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)
Blocking Solution (protein blocking)	• **Reagent 1** • 5 min at room temperature (*This step is optional*)
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052) (*This step is necessary if the protein block has been applied.*)
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.
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)
Biotinylated Secondary Antibody, polyvalent	• **Reagent 2** • 10-15 min at room temperature
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)
Streptavidin-HRP-Conjugate	• **Reagent 3** • 10-15 min at room temperature
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)
Chromogenic substrate	Chromogen Substrate Kits with DAB: REF: DAB-057, DAB-530 REF: DABPLUS-500, DABPLUS-5000 Chromogen Substrate Kits with AEC: REF: ZUC042-050, ZUC042-500 *Attention: Not included in this Kit!* • prepare ready-to-use Chromogen solution according to instructions for use • 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. • Bluing in warm tap water for ~3 min.
Mounting	• AEC: aqueous • DAB: permanent (via ethanol and xylene or xylene replacement)

Automated procedure in the IntelliPathFLX® device from BioCare Medical	
Parameter	**Zytomed Systems recommendation**
Peroxide blocking	• 3% H_2O_2 solution • 10 min at room temperature
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC66)
Blocking Solution (protein blocking)	• **Reagent 1** • 5 min at room temperature (*This step is optional*)
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC066) (*This step is necessary if the protein block has been applied.*)
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.
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC66)
Biotinylated Secondary Antibody, polyvalent	• **Reagent 2** • 10-15 min at room temperature

INSTRUCTIONS FOR USE

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

Streptavidin-HRP-Conjugate	• **Reagent 3** • 10-15 min at room temperature
Washing	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC052)
Chromogenic substrate	Chromogen Substrate Kits with DAB: REF: DAB-057, DAB-530 REF: DABPLUS-500, DABPLUS-5000 Chromogen Substrate Kits with AEC: REF: ZUC042-050, ZUC042-500 *Attention: Not included!* • prepare ready-to-use Chromogen solution according to instructions for use • 5-15 min, (depending on the desired staining intensity)
Stopping the reaction	• 2x with CE/IVD-labelled wash buffer from Zytomed Systems (e.g. ZUC66)
Counterstaining	• Hematoxylin for 30 sec to 10 min (depending on the desired staining intensity) at room temperature. • Bluing in warm tap water for ~3 min.
Mounting	• AEC: aqueous • DAB: permanent (via ethanol and xylene or xylene replacement)

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 as well as the chromogenic substrate (if not included into the detection kit). By using a suitable detection system, the target antigen can be visualized.

11 Analytical performance characteristics

Analytical performance studies for the parameter precision (including repeatability and reproducability) and analytical specificity were performed. The relevant experiments met the pre-specified acceptance critiera 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, HRP (ZytoChem Plus (HRP) Broad Spectrum 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. 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.
- All components contain 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. A mixture of isothiazolones is used for stabilization. Disposal is 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 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

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