

INSTRUCTIONS FOR USE

Detection system (ready-to-use individual product or kit with ready-to-use and/or with concentrated components)



ZytoChem Plus (HRP) One-Step Polymer anti-Mouse/Rabbit

REF	Description
ZUC053-006	6 ml
ZUC053-100	100 ml

Specifications

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

Intended purpose



The ZytoChem Plus (HRP) One-Step Polymer anti-Mouse/Rabbit is used for the qualitative detection of antigens during immunohistochemistry (IHC) on human formalin-fixed, paraffin-embedded (FFPE) tissue sections. It was developed for use in combination with mono- and polyclonal primary antibodies from mouse and rabbit and 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) One-Step Polymer anti-Mouse/Rabbit 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).

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.

Reagents provided

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

REF	Description	Composition
ZUC053-006	6 ml, ready-to-use 	Goat antibody molecules conjugated to horseradish peroxidase (HRP) originated from horseradish plant extract.
ZUC053-100	100 ml, ready-to-use 	Goat antibody molecules conjugated to horseradish peroxidase (HRP) originated from horseradish plant extract.

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

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 peroxide-blocking solution
- Chromogenic substrate
- Hematoxylin or another counter staining
- Mounting medium
- Where appropriate steamer or water bath
- Where appropriate automated staining system
- FFPE tissue specimen
- Positive and negative control specimens
- Adhesive slides
- Coverslips
- Staining vessels/tanks
- Thermometer
- Timer
- Microscope

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.

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.

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Staining 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 IVD-labelled primary antibodies from Zytomed Systems
- If applicable, IVD-labelled dilution buffer from Zytomed Systems matching the primary antibody
- If applicable, IVD-labelled pre-treatment buffer from Zytomed Systems matching the primary antibody
- IVD-labelled wash buffer from Zytomed Systems
- IVD-labelled chromogenic substrate from Zytomed Systems matching the secondary antibody/polymer
- Immunostainer

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	<ul style="list-style-type: none"> • 3 % H₂O₂ solution • 10 min at room temperature
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Blocking Solution (protein blocking)	<ul style="list-style-type: none"> • Reagent 1 • REF: ZUC007 <i>Attention: Not included!</i> • 5-10 min at room temperature <i>(This step is optional.)</i>
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
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 IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
HRP One-Step Polymer anti-Mouse/Rabbit	30 min at room temperature
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Chromogenic substrate	<ul style="list-style-type: none"> • 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 <i>Attention: Not included!</i> • prepare ready-to-use Chromogen solution according to instructions for use 10 min, control the color development under the light microscope
Stopping the reaction	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Counterstaining	<ul style="list-style-type: none"> • Hematoxylin for 30 sec to 10 min (depending on the desired staining intensity) at room temperature. Bluish in cold tap water for ~3 min.
Mounting	<ul style="list-style-type: none"> • AEC: aqueous DAB: permanent (via ethanol and xylene or xylene replacement)

Automated procedure (intelliPATH FLX® fully automated staining system, BioCare Medical)

Parameter	Zytomed Systems recommendation
Peroxide blocking	<ul style="list-style-type: none"> • 3 % H₂O₂ solution • 10 min at room temperature
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Blocking Solution (protein blocking)	<ul style="list-style-type: none"> • Reagent 1 • REF: ZUC007 <i>Attention: Not included!</i> • 5-10 min at room temperature <i>(This step is optional.)</i>
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
HRP One-Step Polymer anti-Mouse/Rabbit	30 min at room temperature
Washing	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Chromogenic substrate	<ul style="list-style-type: none"> • 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 <i>Attention: Not included!</i> • prepare ready-to-use Chromogen solution according to instructions for use ~10 min (depending on the desired staining intensity)
Stopping the reaction	2x with IVD-marked wash buffer from Zytomed Systems (e.g. ZUC052)
Counterstaining	<ul style="list-style-type: none"> • Hematoxylin for 30 sec to 10 min (depending on the desired staining intensity) at room temperature. Bluish in cold tap water for ~3 min.
Mounting	<ul style="list-style-type: none"> • AEC: aqueous DAB: permanent (via ethanol and xylene or xylene replacement)

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.

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.

Analytical performance characteristics

Analytical performance studies for the parameter precision (including repeatability and reproducibility) and analytical specificity were performed. The 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. Hence, ZUC053 (ZytoChem Plus (HRP) One-Step Polymer anti-Mouse/Rabbit) achieves analytical performance as required by Regulation (EU) 2017/746, Annex I, 9.1(a).

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.

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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 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.

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. In this clinical context, the user is responsible for the validation of the test system.
- 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.

Warnings and precautions

- Read the safety data sheet before using the product.
- Do not use the product if it is damaged.
- 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.
- 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.
- Samples of human origin and therefore contaminated consumables must be disposed of in accordance with regional legal regulations.

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



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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

Changes compared to the previous version

Version	Changes
01	First Release