

INSTRUCTIONS FOR USE

Wash buffer (concentrate)



Zytomed Systems Wash Buffer (20x)

REF	Description
ZUC020-500	500 ml
ZUC020-2500	2500 ml

1 Specifications

For the performance of washing steps in the context of IHC on FFPE tissue sections.

2 Intended purpose

The Zytomed Systems Wash Buffer (20x) is a 20-fold concentrated rinsing solution that is suitable for removing excess antibody, enzyme or staining solutions. It is intended to be used in qualitative immunohistochemistry (IHC) on human formalin-fixed, paraffin-embedded (FFPE) tissue sections. The product is intended for professional laboratory use by qualified personnel. Zytomed Systems Wash Buffer (20x) has been tested 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 immunohistochemical 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
ZUC020-500	500 ml, 20-fold concentrated	Phosphate buffer with salts and detergent, mixture of isothiazolones for stabilization
ZUC020-2500	2500 ml, 20-fold concentrated	Phosphate buffer with salts and detergent, 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)
- 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
- Chromogenic substrate
- 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 room temperature (18-25 °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. The stability of the working solution must be validated by the user.

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. 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 secondary antibody or polymer from Zytomed Systems matching the primary antibody
 - 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 buffer, the test system, and the protocol used in the respective clinical context.

Please follow the recommendations below for the staining procedure.

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Parameter	Zytomed Systems recommendations	
	REF	Dilution
Preparing the Working dilution	ZUC020-500	1:20 with distilled or deionized water
	ZUC020-2500	1:20 with distilled or deionized water
pH-value of working dilution	7.2 (7.0 - 7.4) Note: Measure pH value and adjust with diluted NaOH or HCl if necessary.	

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 detection system. The use of a suitable wash buffer optimizes the rinsing of the reagents used between the individual steps of the protocol and thus reduces unspecific staining.

11 Analytical performance characteristics

Analytical performance studies for the parameter precision (including repeatability and reproducibility) were performed. Each experiment met the pre-specified acceptance criteria of at least 90 % positive and negative percent agreement (PPA / NPA). Hence, ZUC020 (Wash Buffer (20x)) achieves analytical performance as required by Regulation (EU) 2017/746, Annex I, 9.1(a), 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.
- 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

- Elias JM "Immunohistopathology – A practical Approach to Diagnosis" ASCP Press 2003
- Nadji M and Morales AR. Ann N.Y. Acad Sci 420:134-139, 1983



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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:



GSH07: Warning / Attention

RUO

For Research Use Only

18 Changes compared to the previous version

Version	Changes
02	<ul style="list-style-type: none"> Adaptation to current template V04 Addition of notes in section 15 General editorial corrections