ZETA Corporation

Instructions for Use (IFU)

B Cell Clonality Cocktail (ZR472+LcN2) Rabbit & Mouse Monoclonal Antibody Cocktail

For *In Vitro* Diagnostic Use (IVD)

Product Identification

Z2837MRP 7 mL (pre-dilute)

Intended Use

This antibody cocktail is intended for *in vitro* diagnostic (IVD) use. B Cell Clonality Cocktail (ZR472+LcN2) Rabbit & Mouse Monoclonal Primary Antibody Cocktail is intended for professional laboratory use in the detection of the Kappa & Lambda protein in formalin-fixed, paraffin-embedded tissue stained in manual and automated qualitative immunohistochemistry (IHC) testing.

This antibody cocktail is intended to be used after the primary diagnosis of the tumor has been made by conventional histopathology using non-immunological histochemical stains. A qualified pathologist must interpret the results using this product to aid diagnosis in conjunction with the patient's relevant clinical history, other diagnostic tests, and proper controls.

Summary and Explanation

Zeta's B Cell Clonality Cocktail provides dual staining for kappa and lambda light chains in immunohistochemistry (IHC), which is a tool used in assessing B-cell clonality to help differentiate reactive (polyclonal) lymphoid proliferations from neoplastic (monoclonal) B-cell processes, such as B-cell lymphomas.

B cells are a type of white blood cell that plays a key role in the immune system by producing antibodies. Clonality refers to the process where a single B cell undergoes division to produce a population of identical cells- plasma cells, which produce monoclonal immunoglobulin with either kappa or lambda light chain. In normal lymphoid tissue, the kappa and lambda cell ratio are approximately 0.3-1.7. In the hematological diseases, such as B-cell lymphomas or B-cell leukemias, there is presence of abnormal monoclonal B cell population with either kappa light chain (>1.7) or lambda light chain (<0.3) predominance. With AP-labeled kappa (ZR471) (red) and HRP labeled lambda (LcN2) (brown) paraffin immunohistochemistry, the B cell/plasma cell clonality can be established.

Principle of Method

B Cell Clonality Cocktail (ZR472+LcN2) Rabbit & Mouse Monoclonal Primary Antibody Cocktail is used with formalin-fixed and paraffin-embedded sections. Pretreatment of deparaffinized tissue with heat-induced epitope retrieval or enzymatic retrieval is recommended.

In general, immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of specific antibodies to the antigen (primary antibodies), a secondary antibody that is isotype-specific to the primary antibody (link antibody), an enzyme complex, and chromogenic substrates with interposed washing steps. The enzymatic activation of the chromogens results in a visible reaction product at the antigen site. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Materials Provided

B Cell Clonality Cocktail (ZR472+LcN2) Rabbit & Mouse Monoclonal Cocktail in prediluted form.

Antibody Cocktail Specifications:

Antibodies as purified antibodies diluted in Tris-HCl buffer containing stabilizing protein and <0.1% ProClin.

Host: Rabbit & Mouse

Isotype: IgG + IgG2a/k

Immunogen: Synthesized human Kappa & Lambda protein

Cellular Localization: Cytoplasmic

Concentrate Dilution Range: Ready-to-use

Positive control: Lymph node

Storage and Handling

Upon receiving, store the vial at 2-8°C. When stored at 2-8°C, this antibody cocktail is stable for 18 months.

To ensure proper reagent stability and functionality, immediately after use the cap must be replaced, and the bottle must be placed in a refrigerator in an upright position. Do not use it after the expiration date is stamped on the vial. If reagents are stored under conditions other than those specified in the package insert, the user must validate them. Repeat freeze and thaw cycles may decrease antibody activity and should be avoided.

If packaging or contents appear broken or damaged, do not use them and contact Zeta Corporation. Contact information is on the last page of this document.

Reconstitution

Predilute Antibodies: Ready to use; no reconstitution necessary.

Refer to the label for any specific instructions.

Stability after dilution: 7 days at 24°C, three months at 2-8°C, and six months at -20°C as demonstrated by stability studies performed at Zeta Corporation when using polypropylene containers.

Materials Required but not Provided

1. Positive Tissue Control: Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded Lymph node
2. Negative tissue control (internal or external)
3. Microscope slides and coverslips
4. Staining jars or baths

5. Timer
6. Xylene or xylene substitute
7. Ethanol or reagent alcohol
8. Deionized or distilled water
9. Heating equipment or enzyme for tissue pretreatment step
10. Detection system
11. Chromogens
12. Wash Buffer
13. Hematoxylin
14. Antibody diluents
15. Peroxide Block
16. Light Microscope
17. Mounting medium
18. Avidin-Biotin Blocking Reagents for use with streptavidin-biotin detection

Specimen Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, and paraffin-embedded tissue sections are used with this primary antibody cocktail. Approximately 4 µm tissue sections are preferred and should be placed on positively charged slides. Pretreatment of deparaffinized tissue with heat-induced epitope retrieval is recommended. Results may vary due to prolonged fixation, unexpected foreign materials, or interfering substances. Decalcification may also affect the staining results.

Tissues from persons infected with the hepatitis B virus containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.

Immunohistochemistry protocol

1. Deparaffinization.
2. Rehydration.
3. Epitope Retrieval Technique: Zeta Tris-EDTA HIER Solution pH 9.0 (ZD6) at 100℃ for 40 minutes using a pressure cooker. Refer to the commercially available device data sheet for specific instructions.
4. Wash with Zeta TBS (ZD5).
5. Peroxide block: Block for 5 minutes at ambient temperature.
6. Rinse with deionized water. Wash with Zeta TBS (ZD5).
7. Primary antibody cocktail: Incubate for 40 minutes at ambient temperature.
8. Wash with Zeta TBS (ZD5).
9. Zeta Dual AP/HRP Detection Polymer (ZD9): Incubate for 40 minutes at ambient temperature.
10. Wash with Zeta TBS (ZD5). Rinse with deionized water.
11. DAB Chromogen: Incubate for 5 minutes at ambient temperature.
12. Wash with Zeta TBS (ZD5)
13. AP Chromogen (ZD16): Incubate for 20 minutes at ambient temperature.
14. Rinse with deionized water.
15. Counterstain with hematoxylin. Rinse with deionized water.

Warnings and Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (for example: xylene).
2. Avoid contact with reagents with eyes and mucous membranes. If reagents contact sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. The user must validate incubation times and temperatures.
5. The prediluted, ready-to-use reagents are optimally diluted, and further dilution may result in loss of antigen staining.
6. The concentrated reagents may be diluted optimally based on validation by the user. Any diluent used that is not explicitly recommended herein must likewise be validated by the user for compatibility and effect on stability.
7. This product is not classified as hazardous when used according to instructions. The preservative in the reagent is less than 0.1% ProClin and does not meet the OSHA (USA) criteria for the hazardous substance at the stated concentration. Refer to Zeta Corporation's website's Safety Data Sheet (SDS).
8. The user must validate storage conditions other than those specified in the package insert.
9. Diluent may contain bovine serum albumin, and supernatant may contain bovine serum. The products containing fetal bovine serum and bovine serum albumin are purchased from commercial suppliers. The certificates support that the bovine sources are from countries with negligible BSE risk and state bovine sources from the USA and Canada.
10. As with any product derived from biological sources, proper handling procedures should be used.

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain positive and negative staining cells or components and serve as positive and negative control tissue. External Positive control materials should be fresh autopsy/ biopsy/ surgical specimens fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared

tissues and proper staining methods. The tissues used for the external positive control materials should be selected from the patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed to detect subtle changes in the primary antibody cocktail's sensitivity from instability or problems with the staining methodology. A tissue with weak positive staining is more suitable for optimal quality control and detecting minor reagent degradation levels.

Negative Tissue Control

Internal or external negative tissue control may be used depending on the organization's guidelines and policies to which the end-user belongs.

The variety of cell types in many tissue sections offers internal negative control sites, but the user should verify this. The components that do not stain should demonstrate the absence of specific staining and indicate non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Interpretation of Results

The immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibodies. Refer to the appropriate detection system package insert for expected color reactions. A licensed pathologist experienced in immunohistochemistry procedures must evaluate the control tissues before interpreting results.

Positive Tissue Control

The stained positive control should be examined first to ensure all reagents function properly. The presence of an appropriately colored reaction product within the target cells or markers indicates positive reactivity. Refer to the package insert of the detection system used for color reactions. Excessive or incomplete staining may compromise the proper interpretation of results. If positive tissue control fails to demonstrate appropriate positive staining, any results with the specimens are considered invalid.

Negative Tissue Control

The negative tissue control (internal or external) should be examined to verify the primary antibody cocktail's specific labeling of the target antigen after the positive tissue control. The absence of specific staining in the negative tissue control confirms the lack of the antibody cocktail's cross-reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid.

Patient Tissue

Patient tissues should be examined next. Positive staining should be assessed within the context of any background staining of the (internal) negative control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false-negative reactions. A qualified pathologist must interpret the patient's morphologic findings and relevant clinical data.

Limitations

1. This reagent is for professional laboratory use only as immunohistochemistry is a multiple-step process that requires specialized training in selecting the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
2. For *in vitro* diagnostic use.
3. Tissue staining is dependent on the handling and processing of the tissue before staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false-negative results. Inconsistent results may result from variations in fixation and embedding methods and inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise the proper interpretation of results.
5. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria, and other diagnostic tests. This antibody cocktail is intended to be used in a panel of antibodies if applicable. A qualified pathologist must be familiar with the antibodies, reagents, diagnostic panels, and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Zeta provides antibodies in concentrated and prediluted formats at optimal dilution. Please refer to Dilution Range and Reconstitution on pages 1 and 2.
   Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users, in any circumstance, must accept responsibility for interpreting patient results.

7. Zeta provides some antibodies in a concentrated format so that the user may subsequently optimally dilute them for use, subject to the user's determination and adherence to suitable validation techniques. Users must validate the use of diluents other than what is recommended herein (see Dilution Range). Users, in any circumstance, must accept responsibility for interpreting patient results.
8. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unforeseen reactions, even in tested tissue groups, cannot be eliminated because of the biological variability of antigen expression in neoplasms or other pathological tissues.
9. Tissues from persons infected with the hepatitis B virus containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
10. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of autoantibodies or natural antibodies.
11. False-positive results may be seen because of the non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (for example, liver, brain, breast, or kidney) subject to the type of immunostaining technique used.
12. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.
13. The prediluted antibody cocktail products are optimized as ready-to-use products. Because of the possibility of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody cocktail incubation time on individual specimens.
14. Combined with detection systems and accessories, this antibody cocktail detects antigen(s) that survive routine formalin fixation, tissue processing, and sectioning. Users who deviate from recommended test procedures *remain, as they would in any circumstance, responsible for interpreting and validating* patient results.
15. For laboratory use only.

Performance Characteristics

B cell/plasma cell clonality

Tissue Types	B Cell Clonality Cocktail Positive (%)
Reactive lymphoid tissue	~95–99%
B-cell lymphoma (overall)	~60–85%

Excerpt: Guo L et al. Mod Pathol. 2018 Mar;31(3):385-394.

The intended purpose, characteristic, and outcome of each antibody cocktail are unique, and therefore, the results of staining should be assessed and interpreted appropriately by a licensed pathologist.

References

1. Bray M, et al: Am J Clin Pathol. 1983; 80(4):526-8
2. Falini B, et al. J Histochem Cytochem. 1982; 30(1):21-6.

Please consult the manufacturer or competent authority if the user experiences technical or performance-related issues. Any severe incident about the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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Symbol Glossary

Symbol	Definition
	Expiration Date
LOT	Lot Number
REF	Catalog Number
	Consult Instructions for Use
	Temperature Limit

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