ZETA Corporation

ALK (1A4) Mouse Monoclonal Antibody

For In Vitro Diagnostic Use (IVD)

Product Identification

Z2289ML	1.0 ml (Concentrate)
Z2289MS	0.5 ml (Concentrate)
Z2289MT	0.1 ml (Concentrate)
Z2289MP	7 mL (pre-dilute)

Intended Use

This antibody is intended for *in vitro* diagnostic (IVD) use.
ALK (1A4) Mouse Monoclonal Primary Antibody is intended for laboratory professional use in the detection of the Anaplastic lymphoma kinase (ALK) in formalin-fixed, paraffin-embedded tissue stained in manual qualitative immunohistochemistry (IHC) testing.
This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using non-immunological histochemical stains. The results using this product must be interpreted by a qualified pathologist as an aid to diagnosis in conjunction with the patient's relevant clinical history, other diagnostic tests and proper controls.

Summary and Explanation

The ALK gene encodes a receptor tyrosine kinase, which belongs to the insulin receptor superfamily. It plays an important role in the development of the brain and exerts its effects on specific neurons in the nervous system. This gene has been found to be rearranged, mutated, or amplified in a series of tumors including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer. The chromosomal rearrangements are the most common genetic alterations in this gene, which result in creation of multiple fusion genes in tumourigenesis, including ALK/EML4, ALK/RANBP2, ALK/ATIC, ALK/TFG, ALK/NPM1, ALK/SQSTM1, ALK/KIF5B, ALK/CLTC, ALK/TPM4, and ALK/MSN. The specificity of the anti-human ALK antibody (clone 1A4)was established on known ALK positive and negative non-small cell carcinoma (NSCLC). The anti-ALK presented no staining on formalin fixed ALK negative NSCLC tissue and positive staining on FFPE ALK positive NSCLC tissue using immunohistochemistry.

Principle of Method

ALK (1A4) Mouse Monoclonal antibody 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 a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link antibody), an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen 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

ALK (1A4) Mouse Monoclonal in concentrated form or prediluted
Antibody Specifications:
Antibody as Purified antibody diluted in Tris-HCl buffer containing stabilizing protein and <0.1% ProClin
Host: Mouse
Isotype: IgG2b /κ
Immunogen: Human protein fragment corresponding to amino acids 1300-1620 of human ALK produced in E. coli
Cellular Localization: Cytoplasmic
Concentrate Dilution Range: 1:30-50
Positive control: Lung adenocarcinoma with ALK translocation

Storage and Handling

Upon receiving, store vial at 2-8°C. When stored at 2-8°C, this antibody is stable for 24 months.
To ensure proper reagent stability and functionality, the cap must be replaced and the bottle must be placed in a refrigerator immediately in an upright position. Do not use after the expiration date stamped on the vial.
If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.
Repeat freeze and thaw cycles may decrease antibody activity and should be avoided.
If packaging or contents appear to be broken or damaged do not use and contact Zeta Corporation. Contact information is on the last page of this document.

Reconstitution:

Predilute Antibodies: Ready to use, no reconstitution necessary.
Concentrate Antibodies: Refer to established dilution range 1:30-50 and use appropriate lab-standardized diluent and container. Refer to the label for any specific instructions.
Stability after dilution: 7 days at 24°C, 3 months at 2-8°C, 6 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 Lung adenocarcinoma with ALK translocation.
2. Negative control tissue (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. Chromogen
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 section tissues are used with this primary antibody. Approximately 4um tissue sections are preferred and they 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 or unexpected introduction of foreign materials or interfering substances. Decalcification may also affect the staining results.
Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.

Warnings and Precautions

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with 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 user. Any diluent used must likewise be validated by the user for both its compatibility and effect on stability.
7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% ProClin and does not meet the OSHA (USA) criteria for hazardous substance at the stated concentration. Refer to the Safety Data Sheet (SDS) on Zeta Corporation's website.
8. The user must validate any 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 products containing bovine serum albumin are purchased from commercial suppliers. The certificates support that the bovine sources are from countries with negligible BSE risk and state sources of bovine from 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 both positive and negative staining cells or tissue components and serve as both the 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 ensure detection of subtle changes in the primary antibody sensitivity from instability or problems with the staining methodology. A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation.

Negative Control Tissue

Internal or external negative control tissue may be used depending on the guidelines and policies that govern the organization to which the end user belongs to.
The variety of cell types present in many tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should

demonstrate the absence of specific staining, and provide an indication of 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 antibody. 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 that all reagents are functioning 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 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 after the positive tissue control to verify the specific labeling of target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody 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. The patient's morphologic findings and relevant clinical data must be interpreted by a qualified pathologist.

Limitations

1. This reagent is for laboratory professional use only as immunohistochemistry is a multiple step process that requires specialized training in the selection of 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 prior to 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, as well as from inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise 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 as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies if applicable. It is the responsibility of a qualified pathologist to 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 both concentrated and prediluted formats at optimal dilution for use. 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 interpretation of patient results.
7. Zeta provides some antibodies in concentrated format so that the user may subsequently optimally dilute for use subject to the user's determination of and adherence to suitable validation techniques. Users must validate the use of any diluents other than what is recommended herein (see Dilution Range). Users in any circumstance must accept responsibility for interpretation of patient results.
8. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.

9. Tissues from persons infected with hepatitis B virus and 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 the effect of autoantibodies or natural antibodies.
11. False positive results may be seen because of 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 (example: liver, brain, breast, 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 products are optimized as a ready-to-use product. Because of the possibility of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
14. In combination with detection systems and accessories, this antibody detects antigen(s) that survive routine formalin fixation, tissue processing and sectioning. Users who deviate from recommended test procedures *herein* remain, as they would in any circumstance, responsible for interpretation and validation of patient results.
15. For laboratory use only.

Performance Characteristics

Lung adenocarcinoma with ALK translocation

Tissue Types	ALK Positivity (%)
Dedifferentiated liposarcoma	3/4 (75)
Myxoid liposarcoma	12/17 (71)
Inflammatory myofibroblastic tumor	68/134 (51)
Well differentiated liposarcoma	9/19 (47)
Rhabdomyosarcoma	10/38 (26)
Myxofibrosarcoma	2/10 (20)
Malignant fibrous histiocytoma	7/38 (18)
Schwannoma	1/5 (20)
Malignant peripheral nerve sheath tumor	3/22 (14)
Synovial sarcoma	1/23 (4)
Leiomyosarcoma	4/28 (4)
Desmoid type fibromatosis	0/30 (0%)
Fibrosarcoma	0/11 (0%)
Neurofibroma	0/3 (0%)
Solitary fibrous tumor	0/3 (0%)

Reference: Chu, Peiguo, and Lawrence M Weiss. Modern Immunohistochemistry. 2nd ed., Cambridge, Cambridge University Press, 2014, pp.1-479.

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

References

1. S, van den Borne BE, et al. ClinCancerRes.2017; 23:4251-425.
2. Shi R, et al. J Thorac Oncol. 2016;11:2248-2252.
3. Rooper LM, et al. Hum Pathol. 2016; 51:139-45.

In the event that the user experiences any technical or performance-related issues with the product, please consult the manufacturer or a competent authority.

Any serious incident that has occurred in relation to 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
C	Concentrate
P	Predilute
S	Supernatant
DIL	Dilution

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IVD

EC REP

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