ALK [5A4]
Concentrated and Prediluted Monoclonal Antibody
901-3041-072717

Intended Use:
For In Vitro Diagnostic Use
ALK [5A4] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of ALK protein or ALK-NPM chimeric protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
ALK (p80) recognizes the formalin-resistant epitope of native anaplastic lymphoma kinase (ALK) protein. ALK recognizes a 200 kDa transmembrane molecule expressed only in neural tissues. The ALK reacts with normal ALK protein, as well as with the chimeric protein ALK-NPM. ALK specifically labels (2;5)-positive cells giving strong cytoplasmic staining that is also associated with nuclear staining. Anaplastic large cell lymphoma (ALCL) is a heterogeneous group of diseases by morphology, immunophenotyping and clinical presentation. It can be difficult to diagnose because of its similarity to Hodgkin’s lymphoma. However, treatment and prognosis of Hodgkin’s and ALCL is very different, and it is imperative to diagnose correctly. Research has also shown that a subset of lung adenocarcinomas harbor rearrangements of the ALK gene that results in the pathologic expression of a fusion protein, most commonly EMLA-ALK. Patients with ALK-rearranged lung adenocarcinomas are unresponsive to tyrosine kinase inhibitors that target EGFR, but have shown dramatic improvement in response to tyrosine kinase inhibitors that target ALK in ongoing clinical trials. The results from studies comparing FISH, CISH and IHC were concordant. The sensitivity and specificity of IHC was reported as 100% and 95% respectively. Based on these findings, the IHC assay using the 5A4 antibody reliably detected non-small cell lung cancer with ALK rearrangement and may be useful as a screening method to identify these tumors. Research has shown that ALK stains the majority of CD30+ ALCL. It has not been shown to stain Hodgkin’s disease (Reed-Sternberg cells). ALK should be used in a panel with CD5, CD20, TIA-1 and EMA.

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested.
Clone: 5A4
Isotype: IgG1
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.
Epitope/Antigen: aa 419-520 of NPM-ALK transcript
Cellular Localization: Cytoplasmic and nuclear staining (dot-like)
Positive Control: Anaplastic large cell lymphoma
Known Applications: Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations:
Peroxide Block: Block for 5 minutes with Biocare’s Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare’s Borg Decloaker. Refer to the Borg Decloaker product data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare’s Background Punisher.
Primary Antibody: Incubate for 30-60 minutes at RT
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare’s DAB -OR- Incubate for 5-7 minutes at RT with Biocare’s Warp Red.
Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note: This antibody has been standardized with Biocare’s MACH 4 detection system. Use TBS buffer for washing steps.

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titer’s listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:
Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare’s Technical Support at 1-800-542-2002.

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaH3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large
Precautions Cont’d:

volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)

2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)

3. Microbial contamination of reagents may result in an increase in nonspecific staining.

4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

5. Do not use reagent after the expiration date printed on the vial.

6. The SDS is available upon request and is located at http://biocare.net.

References:


