

ALK [5A4]

Concentrated and Prediluted Monoclonal Antibody
901-3041-081619

BIOCARE
M E D I C A L

Catalog Number:	ACI 3041 A, B	API 3041 AA	VLTM 3041 G20
Description:	0.1, 0.5 mL, conc.	6.0 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use
Diluent:	Renoir Red	N/A	N/A

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:

Anaplastic large cell lymphoma (ALCL) is associated with a t(2;5) chromosomal translocation, resulting in *ALK* (anaplastic lymphoma kinase) gene fusing with the *NPM* (nucleophosmin) gene (1). The gene fusion leads to the formation of the chimeric NPM-ALK protein, believed to play a role in lymphomagenesis (2). ALK [5A4] monoclonal antibody recognizes the normal ALK protein, as well as with the NPM-ALK, EML4-ALK (the most common fusion in non-small cell lung cancer [NSCLC]), and all other known pathological ALK fusions (2,3). ALK-targeting tyrosine kinase inhibitors (TKI) have proven to be quite effective therapeutics in *ALK*-rearranged NSCLC patients (4). Determining eligibility for ALK TKI therapy requires rapid, accurate screening to detect *ALK* rearrangement, which can be accomplished by detection of *ALK* fusion protein overexpression by immunohistochemistry (IHC) or *ALK* gene rearrangement by *in situ* hybridization (ISH). Studies comparing fluorescence ISH (FISH), chromogenic ISH (CISH), and IHC showed concordance (5-7). Based on these findings, IHC using the ALK [5A4] antibody may be useful as a screening method to identify *ALK*-rearranged NSCLC patients eligible for ALK TKI therapy.

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. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 5A4

Isotype: IgG1

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: aa 419-520 of NPM-ALK transcript

Cellular Localization: Cytoplasmic and nuclear staining (dot-like)

Positive Tissue 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM3041 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Deparaffinization: Deparaffinize for 8 minutes with Val DePar.

Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.

Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.

Primary Antibody: Incubate for 30 minutes.

Secondary: Incubate for 10 minutes with Val Mouse Secondary.

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 10 minutes with Val Universal Polymer.

Chromogen: Incubate for 5 minutes with Val DAB.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker product data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with 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 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, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS 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 titers 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.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

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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 (NaN₃) 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 volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (8)
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. (9)
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>.

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.

References:

1. Morris SW, *et al.* Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science*. 1994 Mar 4;263(5151):1281-4.
2. Falini B, *et al.* ALK expression defines a distinct group of T/Null lymphomas ("ALK lymphomas") with a wide morphological spectrum. *Am J Pathol*. 1998 Sep; 153(3):875-86.
3. Mino-Kenudson M, *et al.* A novel highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res*. 2010 Mar 1; 16(5):1561-71.
4. Heigener DF, Reck M. Crizotinib. *Recent Results Cancer Res*. 2018;211:57-65.
5. Paik JH, *et al.* Screening of anaplastic lymphoma kinase rearrangement by immunohistochemistry in non-small cell lung cancer: correlation with fluorescence in situ hybridization. *J Thorac Oncol*. 2011 Mar; 6(3):466-72.
6. Kim H, *et al.* Detection of ALK gene rearrangement in non-small cell lung cancer: a comparison of fluorescence in situ hybridization and chromogenic in situ hybridization with correlation of ALK protein expression. *J Thorac Oncol*. 2011 Aug; 6(8):1359-66.
7. McLeer-Florin A, *et al.* Dual IHC and FISH testing for ALK gene rearrangement in lung adenocarcinomas in a routine practice: a French study. *J Thorac Oncol*. 2012 Feb; 7(2):348-54.
8. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
9. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.