

CONFIRM anti-ALK1 (ALK01) Primary Antibody

REF 800-2918
05278783001

IVD Σ 50

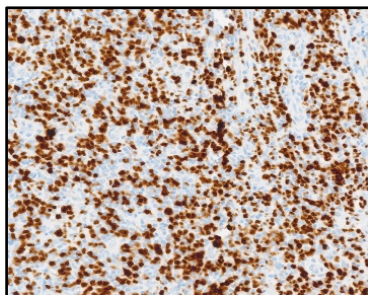


Figure 1. CONFIRM anti-ALK1 (ALK01) antibody staining of inguinal lymph node and positive for Anaplastic Large Cell Lymphoma.

SUMMARY AND EXPLANATION

The human anaplastic lymphoma kinase (ALK) gene encodes a 200 kDa receptor tyrosine kinase protein, which belongs to the insulin receptor superfamily, and is located on chromosome 2 (2p23).^{1,2} The ALK protein is considered a catalytic receptor, a cell-surface protein that binds to its ligand as a cell-surface receptor, which plays an important role in transmembrane signaling and intercellular communication.³ Under normal physiological conditions, ALK is highly expressed in neural cells, primarily during fetal development, and is implicated in neural development and differentiation.⁴⁻⁶ ALK expression in normal adult tissues is limited to few cell types in the nervous system (e.g. neural cells, pericytes, and endothelial cells), although the precise physiological role is not understood.^{5,7}

Anaplastic large cell lymphoma (ALCL) is a non-Hodgkin T-cell lymphoma that occurs as widespread systemic disease or localized cutaneous disease.⁸⁻¹⁰ ALCL is defined by the strong expression of CD30 and various T-cell antigens, although expression of pan T-cell markers is often incomplete.^{10,11} Cases of systemic ALCL are classified into two distinct clinicopathologic entities based on the presence or absence of ALK.^{9,10} ALK-positive ALCL expresses the ALK protein due to ALK gene rearrangements, whereas ALK-negative ALCL, a morphologically indistinguishable entity, lacks these characteristics.^{9,11} The t(2;5)(p23;q35) chromosomal translocation results in the formation of an 80 kDa chimeric protein comprised of the nucleophosmin (NPM) gene and the ALK gene (i.e. NPM-ALK) fusion product.¹ Although NPM is the predominant fusion partner in 80-90% of ALCL cases, more than 25 ALK rearrangement partners have been identified and multiple ALK chimeric proteins (e.g. TMP3-ALK, ATIC-ALK, TFG-ALK) have been observed in ALCL.⁶⁻⁹

The detection of ALK by immunohistochemistry (IHC) with the CONFIRM anti-ALK1 (ALK01) Primary Antibody may be used to aid in the diagnosis of ALK-positive or ALK-negative ALCL.

The cellular staining pattern is cytoplasmic and nuclear in most tumors with the NPM-ALK translocation but is nuclear-restricted in the small-cell variant and usually cytoplasmic in tumors with other translocation variants.⁹ This antibody may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-ALK1 (ALK01) Primary Antibody binds to the ALK protein in formalin-fixed, paraffin-embedded tissue sections. This antibody can be visualized using *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001) or *OptiView* DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-ALK1 (ALK01) Primary Antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-ALK1 (ALK01) Primary Antibody contains approximately 25 µg of a mouse monoclonal antibody.

The antibody is diluted in phosphate buffer containing carrier protein and Proclin 300, a preservative.

Specific antibody concentration is approximately 5 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-ALK1 (ALK01) Primary Antibody is a mouse monoclonal antibody produced as a tissue culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided :

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *OptiView* DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. *OptiView* Amplification Kit (Cat. No. 760-099 / 06396518001 (50 test) or 860-099 / 06718663001 (250 test))
6. *ultraView* Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
7. Amplification Kit (Cat. No. 760-080 / 05266114001)
8. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
9. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
10. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
11. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
12. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
13. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
14. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
15. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
16. General purpose laboratory equipment
17. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8° C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed formalin fixed, paraffin embedded (FFPE) tissues are suitable for use with this primary antibody when used with VENTANA detection kits and a Benchmark IHC/ISH. The recommended tissue fixative is 10% neutral buffered formalin.¹² Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.


It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic (IVD) use.
- For professional use only.
- CAUTION:** In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
- Do not use beyond the specified number of tests.
- ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
- Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.^{13,14}
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as this could produce incorrect results.
- For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
- Consult local and/or state authorities with regard to recommended method of disposal.
- Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
- To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
 Warning	H317	May cause an allergic skin reaction
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+ P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on Benchmark IHC/ISH instrument in combination with VENTANA detection kits and accessories. Refer to the tables below for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the Instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 800-2918.

Table 2. Recommended staining protocols for CONFIRM anti-ALK1 (ALK01) Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 64 minutes	ULTRA CC1 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	36 minutes, 37 °C	32 minutes, 36 °C
OptiView AMP H2O2	4 minutes	4 minutes
OptiView AMP Multimer	4 minutes	4 minutes
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

^aConcordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocols for CONFIRM anti-ALK1 (ALK01) Primary Antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 60 minutes	ULTRA CC1 64 minutes, 95 °C
Antibody (Primary)	32 minutes, 37 °C	48 minutes, 36 °C
Amplification	Selected	Selected
<i>ultra</i> Block	8 minutes	8 minutes
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

^aConcordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."¹⁵

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-ALK1 (ALK01) Primary Antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of a positive control tissue for this antibody is ALK-positive anaplastic large cell lymphoma.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-ALK1 (ALK01) Primary Antibody is mostly cytoplasmic and nuclear, but membranous can be seen less frequently.⁹

SPECIFIC LIMITATIONS

OptiView detection system is generally more sensitive than the *ultra*View detection system. The user must validate the results obtained with this reagent and detection systems.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-ALK1 (ALK01) Primary Antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/4	Esophagus	0/3
Cerebellum	0/4	Stomach	0/3
Adrenal gland ^a	0/4	Small intestine	0/4
Ovary	0/3	Colon	0/4
Pancreas	0/3	Rectum	0/2
Parathyroid gland	0/4	Liver	0/3
Pituitary gland	0/4	Kidney	0/5
Testis	0/4	Prostate	0/3
Thyroid	0/3	Bladder	0/3
Salivary gland	0/3	Ureter	0/2
Breast	0/4	Endometrium	0/3
Spleen ^b	0/6	Cervix	0/4
Tonsil ^b	0/9	Placenta	0/3
Lymph Node ^b	0/5	Skeletal muscle	0/3
Thymus	0/3	Skin	0/4
Myeloid (bone marrow)	0/4	Nerve	0/5
Lung	0/4	Spinal Cord	0/1
Heart	0/4	Mesothelium	0/6

^a Tissues evaluated include normal and hyperplastic adrenal gland

^b Tissues evaluated include normal, reactive, inflamed or congestion

Table 5. Sensitivity/Specificity of CONFIRM anti-ALK1 (ALK01) Primary Antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Fibroblastic meningioma (Cerebellum)	0/1
Meningioma (Cerebellum)	0/1
Fibroblastic meningioma (Cerebrum)	0/1
Astrocytoma (Brain)	0/1
Adenocarcinoma (Oral cavity)	0/1
Squamous cell carcinoma (Oral cavity)	0/1
Melanoma (Nasal Cavity)	0/1
Cortical adenoma (Adrenal gland)	0/1
Cortical carcinoma (Adrenal gland)	0/1
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Ovary)	0/1
Endometrioid adenocarcinoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Adenoma (Parathyroid)	0/1
Seminoma (Testis)	0/2
Adenoma (Thyroid)	0/3
Follicular variant of papillary carcinoma (Thyroid)	0/1
Pleomorphic adenoma (Salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	0/1
Fibroadenoma (Breast)	0/2
Invasive ductal carcinoma (Breast)	0/3
Squamous cell carcinoma (Lung)	0/2
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/3
Adenocarcinoma (Stomach)	0/3
Adenoma (Small intestine)	0/1
Adenocarcinoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Adenocarcinoma (Rectum)	0/3
Hepatocellular carcinoma (Liver)	0/3
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Urothelial carcinoma (Bladder)	0/2
Adenocarcinoma (Endometrium)	0/2
Undifferentiated carcinoma (Uterus)	0/1

Pathology	# positive / total cases
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Metastatic breast invasive ductal carcinoma	0/1
Metastatic esophageal squamous cell carcinoma	0/1
Metastatic colonic signet ring cell carcinoma	0/1
Metastatic cancer from G.I. tract (Lung)	0/1
Metastatic Colon Adenocarcinoma (Liver)	0/1
Diffuse Large B-cell Lymphoma	1/45
Follicular lymphoma	0/15
MALT B-cell lymphoma	0/8
Mantle cell lymphoma	0/6
lymphoplasmacytic lymphoma	0/1
non-Hodgkin B-cell lymphoma	0/16
Small lymphocytic lymphoma	0/8
B-cell lymphoma, NOS	0/1
Hodgkin lymphoma	0/8
Extranodal NK/T-cell lymphoma, nasal type	0/2
NK/T-cell lymphoma	0/1
Enteropathy-associated T- cell lymphoma	0/5
Angioimmunoblastic T-cell lymphoma	0/10
Peripheral T-cell lymphoma, NOS	2/29
Anaplastic large cell lymphoma ALK+	18/18
Anaplastic large cell lymphoma ALK-	0/18
non-Hodgkin T-cell lymphoma, NOS	0/2
Lymphoma, NOS	0/21

Precision

Precision studies for CONFIRM anti-ALK1 (ALK01) Primary Antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX and BenchMark ULTRA instruments.
- Between platform precision between the BenchMark GX and BenchMark ULTRA instruments.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-ALK1 (ALK01) Primary Antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

<https://ec.europa.eu/tools/eudamed>

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see [dialog.roche.com](https://www.dialog.roche.com) for definition of symbols used):



Global Trade Item Number



Unique Device Identification



Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
C	<p>Updates to Intended Use, Summary and Explanation, Principle of the Procedure, Material Provided, Materials Required but not Provided, Storage and Stability, Specimen and Preparation, Warnings and Precautions, Staining Procedure, Negative Reagent Control, Positive Tissue Control, Staining Interpretation / Expected Results, Specific Limitations, Performance Characteristics, Analytical Performance, References, and Intellectual Property</p> <p>Added BenchMark GX, ULTRA, and ULTRA PLUS instruments.</p> <p>Added recommended protocols for OptiView DAB IHC Detection Kit and <i>ultra</i>View Universal DAB Detection Kit.</p> <p>Removed recommended protocols for ES and NexES IHC instruments.</p> <p>Removed recommended protocols for <i>VIEW</i> DAB, AEC, Alkaline Phosphatase Red and Enhanced Alkaline Phosphatase Red detection kits.</p>

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