For research use only

IHC staining system

For detection of
lower expression of ALK fusion proteins

ALK

Anaplastic Lymphoma Kinase

N-Histofine® ALK Detection KIT

Code: 417071F Size: 20 tests

N-Histofine® ALK Detection Kit detects anaplastic lymphoma kinase (ALK) proteins in tumor cells in paraffin-embedded tissue specimens by IHC staining and determines presence of such protein expression.

Background

The *ALK* gene was identified in 1994 as a gene fused to the nucleophosmin (*NPM*) gene in anaplastic large-cell lymphoma (ALCL) with t(2;5)(p23;q35) translocation. This gene is located at 2p23 and encodes for a receptor-type tyrosine kinase, which belongs to the insulin receptor family. The ALK protein have a kinase domain in its intracellular domain, and its function is associated with the promotion of cell growth and inhibition of apoptosis.

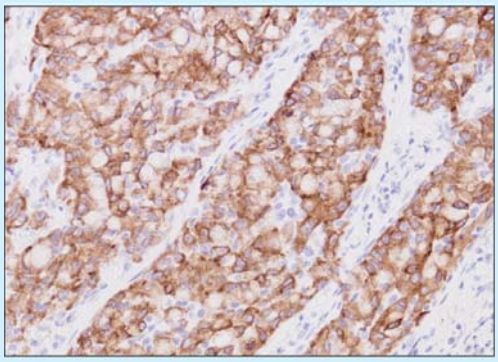
Subsequently, the *ALK* gene has been reported to form *ALK* fusion genes fused with *ATIC, CLTC, MSN, TPM3, TPM4, TFG, MYH9* and *ALO17* genes in ALCL and also *ATIC, CARS, CLTC, DCTN1, TPM3, TPM4, PPFIBP1, RANBP2* and *SEC31L1* genes in inflammatory myofibroblastic tumor (IMT).

The proteins produced from these fusion genes are constantly activated by forming dimers and led to cancerous change.

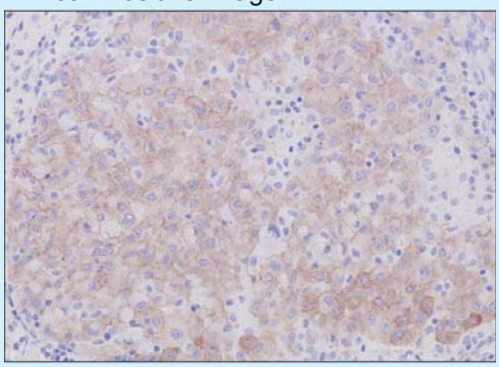
Recently, other *ALK* fusion genes with *EML4* gene, *KIF5B* gene or *KCL1* gene in non-small cell carcinoma of lung, *SEC31A* gene or *SQSTM1* gene in ALK-positive large B-cell lymphoma and *VCL* gene in renal cell cancer have been also reported.

Staining Image

■ Strong Positive Image



■ Weak Positive Image



Lung adenocarcinoma: Positive for cytoplasm of tumor cells. Weak to strong positive of staining levels regarding the expression level of ALK fusion protein. Hot bath treatment (+)

Kit Components

Product Name	Size	Code
N-Histofine® ALK Detection Kit	20 tests	417071F

Vial No.	Components	Constituents	Volume
1	Blocking Reagent	3 V/V% Hydrogen peroxide	4ml × 1
2	Primary Antibody	anti-ALK mouse monoclonal antibody (5A4)	2ml × 1
3	Negative Control	Mouse IgG	2ml × 1
4	Bridge Reagent		4ml × 1
5	Peroxidase Labeled Empower Reagent		4ml × 1
6	Chromogen Substrate	3,3'-Diaminobenzidine tetrahydrochloride	0.5ml × 1
7	Substrate Buffer Solution		0.5ml × 1
8	Chromogen Reagent	0.6 V/V% Hydrogen peroxide solution	0.5ml × 1
9	ALK Antigen Retrieval Solution A		150ml × 1
10	ALK Antigen Retrieval Solution B		150ml × 1

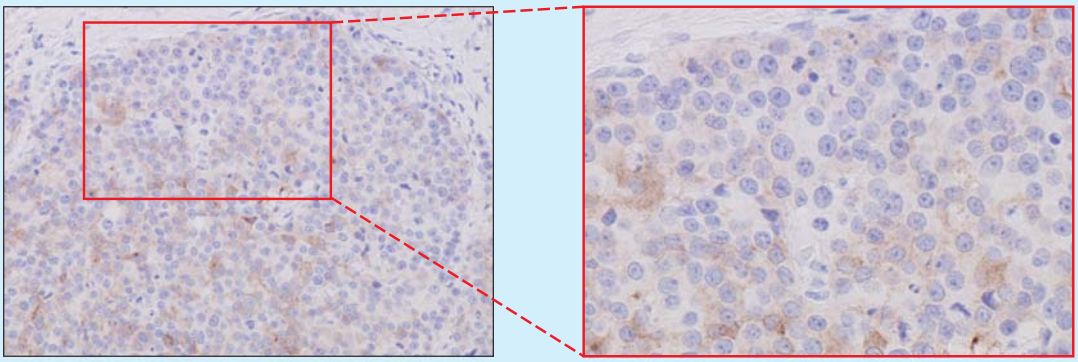
Product Name	Size	Code
N-Histofine® ALK Control Slides	5 slides	417081F

Notes for Determination

Due to this kit detects ALK proteins, ALK fusion proteins as well as full-length ALK protein are reacted. Consequently, results of slight positive to positive for tumors* that infrequently express full-length ALK protein are observed. However, discrimination between ALK fusion proteins and full-length ALK protein is not available. Therefore, considering expression possibility of ALK fusion proteins, confirmation of the presence or absence of *ALK* fusion genes by using FISH method is preferable in this regard.

* Large-cell neuroendocrine carcinomas of the lung, small-cell lung carcinomas and rhabdomyosarcomas (particularly alveolar rhabdomyosarcomas).

■ Weak positive Images in large cell neuroendocrine carcinoma of the lung



Weak positive staining is observed in the specimen which expresses full-length ALK protein.
Hot bath treatment (+)

References

(1) Shiota M., et al: Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 9: 1567-1574, 1994
(2) Morris SW., et al: Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 263: 1281-1284, 1994
(3) Takeuchi K., et al: KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res. 15(9): 3143-3149, 2009
(4) Takeuchi K., et al: Pulmonary inflammatory myofibroblastic tumor expressing a novel fusion, PPFIBP1-ALK: reappraisal of anti-ALK immunohistochemistry as a tool for novel ALK-fusion identification. Clin Cancer Res. 17(10): 3341-3348, 2011
(5) Sugawara E., et al: Identification of Anaplastic Lymphoma Kinase Fusions in Renal Cancer. Cancer, 2012

Manufacturer
NICHIREI BIOSCIENCES INC.
6-19-20, TSUKIJI, CHUO-KU, TOKYO 104-8402, JAPAN
PHONE : 81-3-3248-2207
FAX : 81-3-3248-2243
http://www.nichirei.co.jp/bio/english

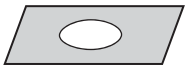
2012.05

N-Histofine® ALK Detection KIT

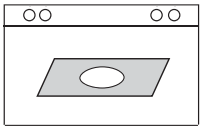
Staining Procedure

- Following procedure is required for accurate staining results.
- Designated temperature and duration of operation is required at each step.
- Operation under RT (15 to 25 °C) is required except designated temperature.

● Preparation of tissue sections



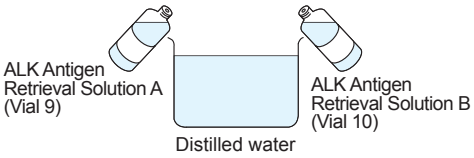
Slice sections into 4 µm thickness and place on poly-L-lysine or silane-coated slides.



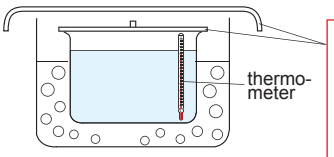
Dry the slides completely in incubator.
(at 37 °C for 24hrs).

●Preparation of ALK Antigen Retrieval Solution

Avoid burn injuries.



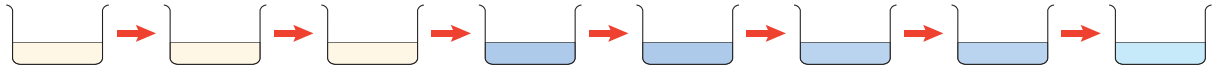
Measure 1 part ALK Antigen Retrieval Solution A to 1 part ALK Antigen Retrieval Solution B to 8 parts distilled water.



Preheat water bath up to 95-99 °C. Prepare ALK Antigen Retrieval Solution in heat-resistant plastic staining jar and heat up to 95-99 °C in the water bath.

Note:
Lids on staining jar and water bath are effective to maintain 95 to 99 °C and to prevent excess water evaporation. Those lids should be placed loosely to avoid completely sealed condition or the jar may have risk of damage.

●Deparaffinization and Rehydration (for paraffin-embedded tissue sections)



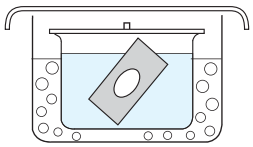
xylene	xylene	xylene	100% ethanol	100% ethanol	95% ethanol	95% ethanol	PBS
3 min.	3 min.	3 min.	3 min.	3 min.	3 min.	3 min.	3 min. x 3

- Remove excess xylene and ethanol at each step.
- Change xylene and ethanol as appropriate to complete deparaffinization and rehydration.

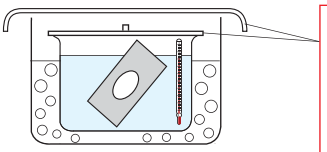
●Antigen Retrieval

Designated temperature and duration of operation is required for accurate staining results.

Avoid burn injuries.

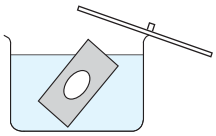


Immerse the slides in 95-99 °C of ALK Antigen Retrieval Solution and place lid on the staining jar loosely.
And place another lid on the water bath loosely then heat the solution up to 95-99 °C again.

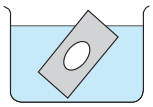


Incubate the slides after confirming temperature of the Solution risen to 95-99 °C by thermometers.
(at 95-99 °C for 40 min.)

Note:
Lids on staining jar and water bath are effective to maintain 95 to 99 °C and to prevent excess water evaporation.
Those lids should be placed loosely to avoid completely sealed condition or the jar may have risk of damage.



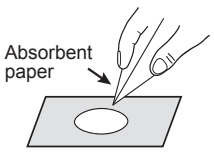
Remove the staining jar from the water bath and uncover the jar. Cool the jar down slowly leaving the slides inside.
(at RT for 20 min.)



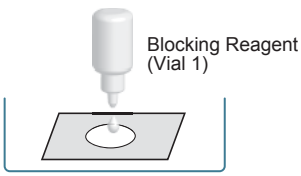
Rinse the slides in PBS.
(at RT for 3 min. x 3)

●Treatment with Blocking Reagent* (Removal of endogenous peroxidase)

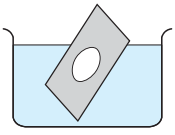
* 3V/V% Hydrogen peroxide solution



Remove PBS around tissue sections.



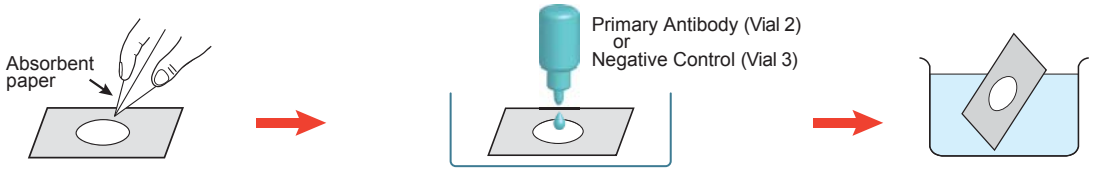
Apply Blocking Reagent to each slide covering sections completely and incubate in moist chamber.
(at RT for 5 min.)



Rinse the slides in PBS.
(at RT for 3 min. x 3)

●Addition and reaction of Primary Antibody* or Negative Control**

* anti-ALK mouse monoclonal antibody (5A4)
** Mouse IgG

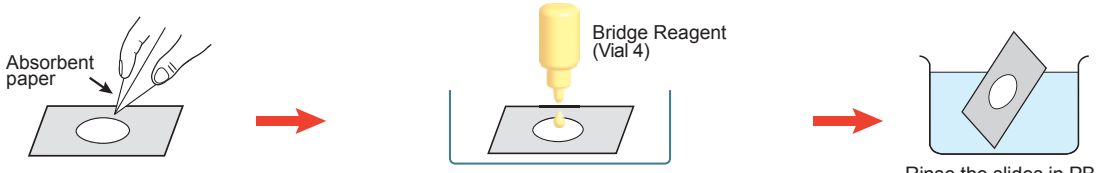


Remove PBS around tissue sections.

Apply Primary Antibody or Negative Control to each slide covering sections completely and incubate in moist chamber. (at RT for 30 min.)

Rinse the slides in PBS. (at RT for 3 min. x 3)

●Addition and reaction of Bridge Reagent

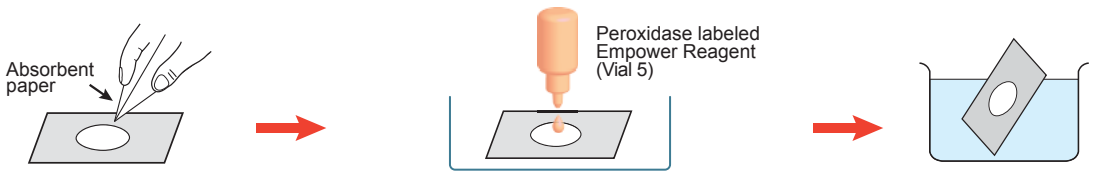


Remove PBS around tissue sections.

Apply Bridge Reagent to each slide covering sections completely and incubate in moist chamber. (at RT for 15 min.)

Rinse the slides in PBS. (at RT for 3 min. x 3)

●Addition and reaction of Peroxidase labeled Empower Reagent

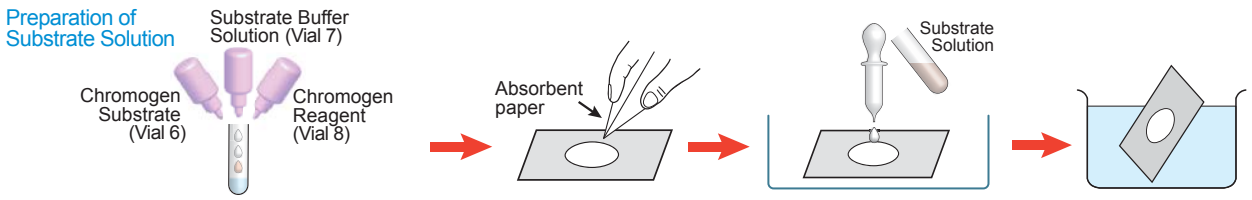


Remove PBS around tissue sections.

Apply Peroxidase labeled Empower Reagent to each slide covering sections completely and incubate in moist chamber. (at RT for 30 min.)

Rinse the slides in PBS. (at RT for 3 min. x 3)

●Preparation, addition and reaction of Substrate Solution



Add each 1 drop of Chromogen Substrate and Substrate Buffer Solution to 1mL of purified water and mix well. And then add Chromogen Reagent to the mixture and mix again. (Use the solution within 30 min. after preparation is recommended.)

Remove PBS around tissue sections.

Apply prepared Substrate Solution to each slide covering sections completely and incubate in moist chamber. (at RT for 10 min.)

Wash the slides with distilled water.

●Counterstaining

Wash the slides with running water after immersed in counterstaining reagent (hematoxylin).

●Mounting

Mount slides with permanent mounting media after dehydration in graded series of alcohol and clearing in xylene.

N-Histofine® ALK Control Slides

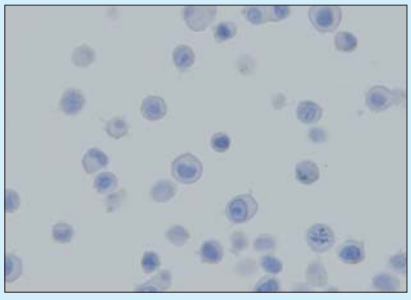
Code: 417081F Size: 5 slides

N-Histofine® ALK Control Slides are used as a standard for discrimination of positive tissue blocks possess expressed ALK protein.

This product is used for validation of reagent performance and staining technique in the IHC staining (including cytology staining) with N-Histofine® ALK Detection Kit.

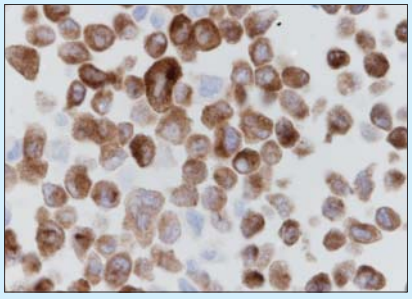
Both formalin fixed paraffin embedded cell lines of NCI-H2228 (Positive) and SK-BR-3 (Negative) are mounted on each slide.

■ **Negative** control cell line/SK-BR-3



No stain is observed in the cytoplasm of all cells. Treatment with hot bath (+)

■ **Positive** control cell line/NCI-H2228



Strong stains are observed in the cytoplasm of the majority of cells. Treatment with hot bath (+)