

For research use only



# IHC staining system

**For determination  
lower expression of ALK fusion proteins**

# ALK

**Anaplastic Lymphoma Kinase**

**N-Histofine<sup>®</sup> ALK Detection Kit**  
**Code: 417071F Size: 20 tests**

This product is an Immunohistochemical (IHC) staining system to determine anaplastic lymphoma kinase (ALK) protein preserved in ALK fusion proteins expressed in tumor cells in paraffin-embedded tissue specimens.

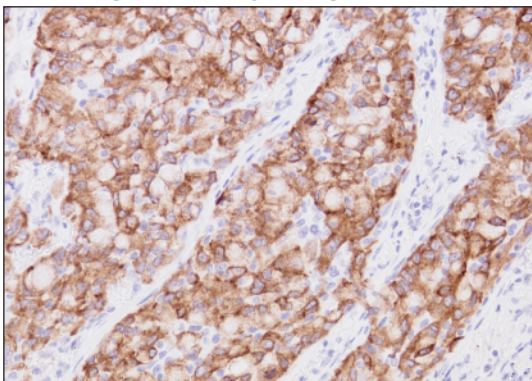
High temperature epitope unmasking is required of formalin fixed paraffin-embedded tissue sections prior to immunostaining.

## Background

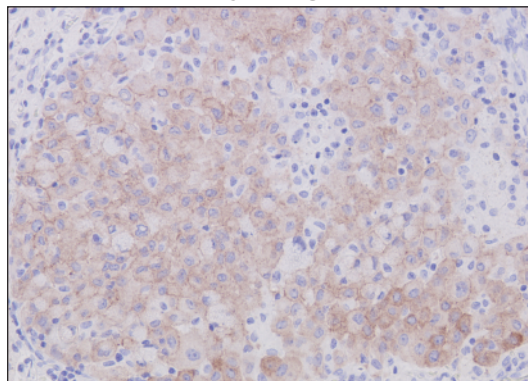
The *ALK* gene was identified in 1994 as a gene fused to the nucleophosmin (*NPM*) gene in anaplastic large-cell lymphoma (ALCL) with t(2;5)(p23;q35) translocation.<sup>(1)(2)</sup> This gene is located at 2p23 and encodes for a receptor-type tyrosine kinase, which belongs to the insulin receptor family. 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. In ALCL and inflammatory myofibroblastic tumor, the *ALK* gene has been reported to fuse with genes such as *TFG*, *ATIC*, *CARS*, *CLTC*, *SEC31L1*, *RANBP2*, and *TPM3*, to form *ALK* fusion genes. The proteins that are produced from these *ALK* fusion genes form dimers, and as a result, the kinase domain of the *ALK* gene was constitutively activated, leading to carcinogenesis. Recently, fusion of the *ALK* gene with several genes in non-small cell lung carcinoma, large B-cell lymphoma, and renal cell carcinoma has been reported. Therefore determination of ALK protein preserved in ALK fusion proteins is useful for ALK studies.

## Staining Image

< Strong Positivity Image >



< Weak Positivity 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

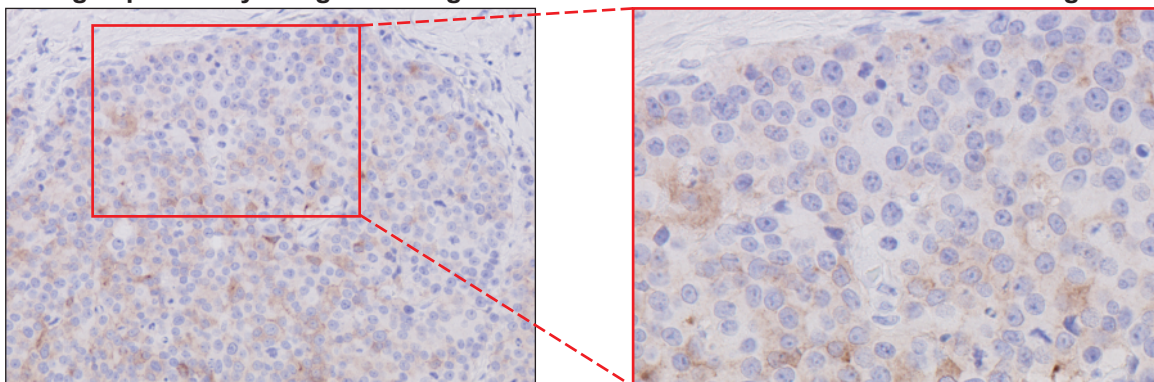
Vial No.	Description 1	Description 2	Quantity
1	Blocking Reagent	3 V/V% Hydrogen peroxide	4ml × 1
2	Primary Antibody	anti-ALK mouse monoclonal antibody (5A4)	2ml × 1
3	Negative Control	Mouse IgG	2ml × 1
4	Bridge Reagent		4ml × 1
5	Peroxidase Labeled Empower Reagent		4ml × 1
6	Chromogen Substrate	3,3'-Diaminobenzidine 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

## Caution

This kit reacts not only with ALK fusion proteins but also with the full-length ALK protein. That is why this kit shows slight positivity or positivity for tumors\*<sup>1</sup> that rarely express the full-length ALK protein. This kit cannot distinguish between ALK fusion proteins and the full-length ALK protein. Therefore, in samples, which show a positivity with this kit, a confirmation of the presence or absence of *ALK* fusion genes by using the fluorescence in situ hybridization (FISH) or RT-PCR method is preferable.

\*1: Large-cell neuroendocrine carcinomas of the lung, small-cell lung carcinomas, and also rhabdomyosarcomas (particularly alveolar rhabdomyosarcomas).

### < Slight positivity Images in large cell neuroendocrine carcinoma of the lung >



Slight positivity 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 oncokinae 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, PPF1B1-ALK: reappraisal of anti-ALK immunohistochemistry as a tool for novel ALK-fusion identification. *Clin Cancer Res*. 17(10): 3341-3348, 2011

Manufacturer

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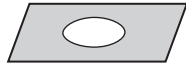
<http://www.nichirei.co.jp/bio/english>

# N-Histofine® ALK Detection Kit

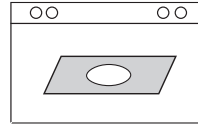
## Staining Procedure

- 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

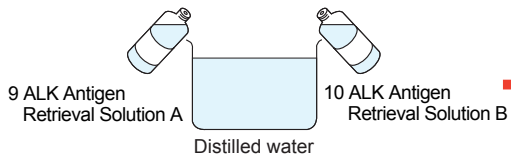


Slice sections into 4 µm thickness and place on poly-L-lysine or silane-coated slides.

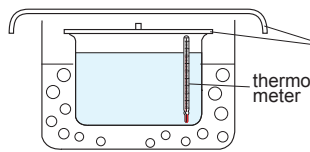


The slides should be dried completely in incubator. (at 37 °C for 24hrs).

### •Preparation of ALK Antigen Retrieval Solution



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



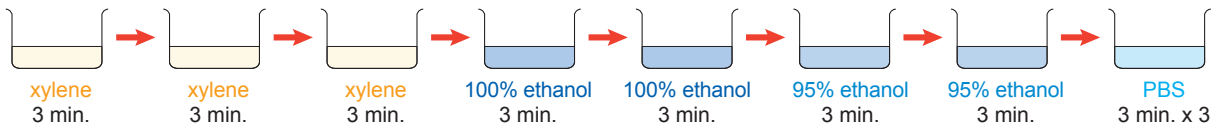
Preheat water bath up to 95-99 °C. Prepare ALK Antigen Retrieval Solution in heat-resistant plastic staining jar and heat up to 95-99 °C in the water bath.

Avoid burn injuries.

**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)

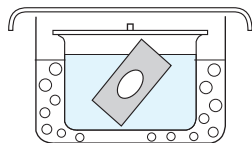


- Remove excess xylene and ethanol at each step.
- Change xylene and ethanol as appropriate to complete deparaffinization and rehydration.

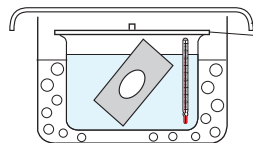
### •Antigen Retrieval

Avoid burn injuries.

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



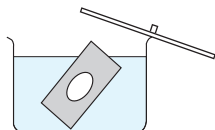
Immerse the slides in 95-99 °C of ALK Antigen Retrieval Solution and place lid on the staining jar loosely. And place another lid on the water bath loosely then heat the solution up to 95-99 °C again.



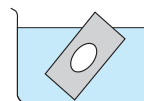
Incubate the slides after confirming temperature of the Solution risen to 95-99 °C by thermometers. (at 95-99 °C for 40 min.)

**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.

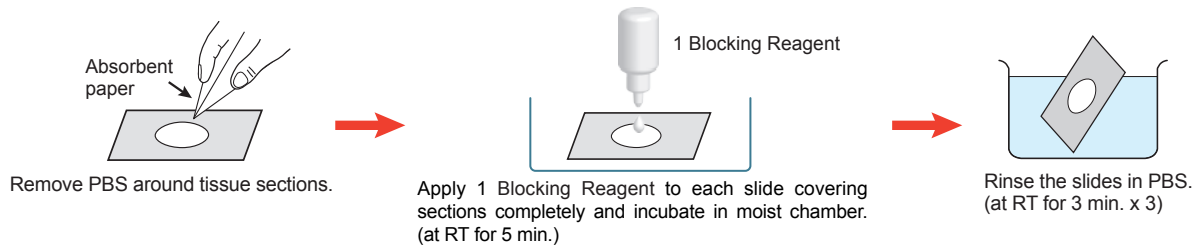


Remove the staining jar from the water bath and uncover the jar. Cool the jar down slowly leaving the slides inside. (at RT for 20 min.)

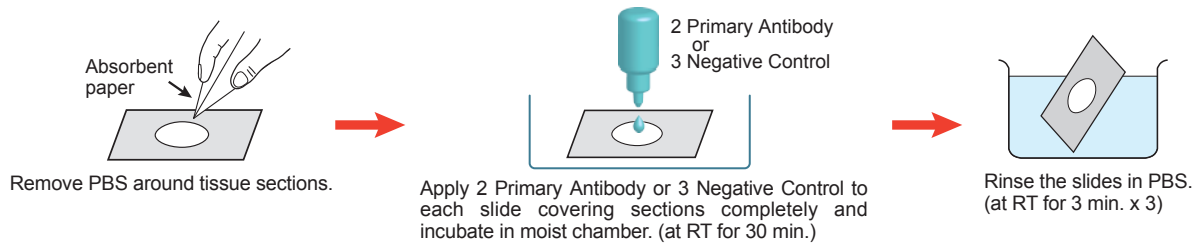


Rinse the slides in PBS. (at RT for 3 min. x 3)

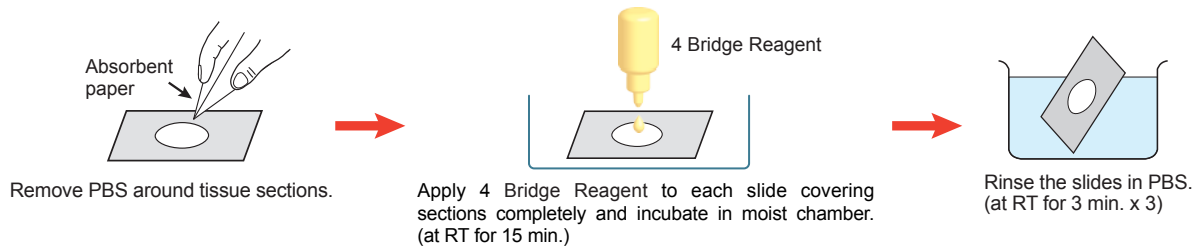
● **Treatment with Blocking Reagent\*** (Removal of endogenous peroxidase) \* 3V/V% Hydrogen peroxide solution



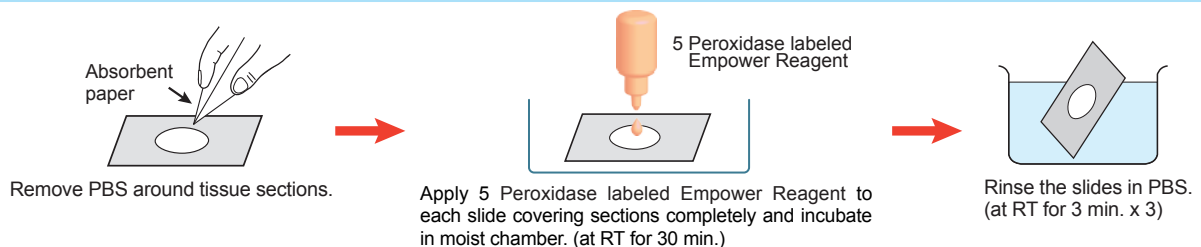
● **Addition and reaction of Primary Antibody\* or Negative Control\*\*** \* anti-ALK mouse monoclonal antibody (5A4) \*\* Mouse IgG



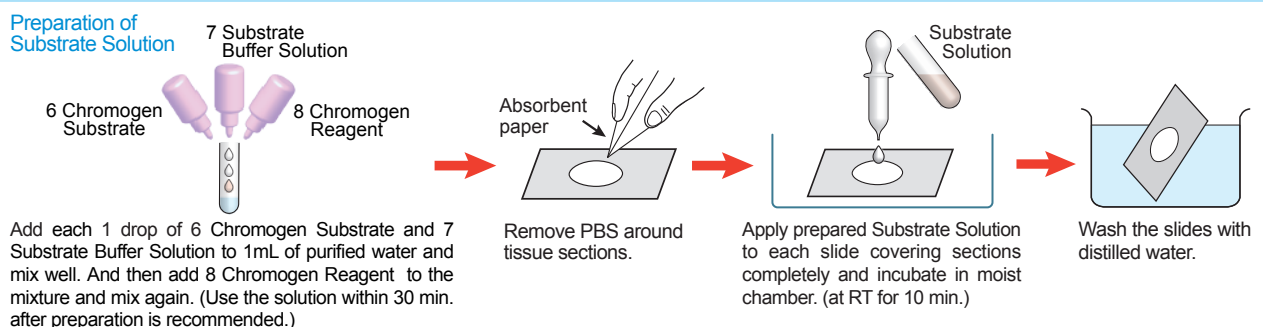
● **Addition and reaction of Bridge Reagent**



● **Addition and reaction of Peroxidase labeled Empower Reagent**



● **Preparation, addition and reaction of Substrate Solution**



● **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.