

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.

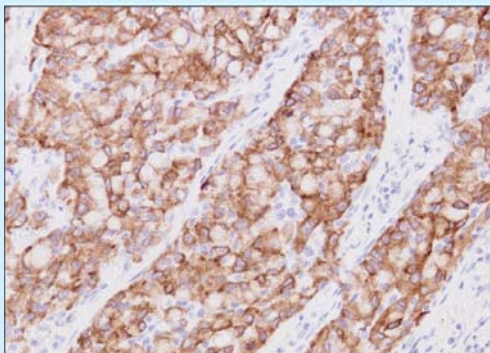
Subsequently, the *ALK* gene has been reported to form *ALK* fusion genes fused with *AT1C*, *CLTC*, *MSN*, *TPM3*, *TPM4*, *TFG*, *MYH9* and *ALO17* genes in ALCL and also *AT1C*, *CARS*, *CLTC*, *DCTN1*, *TPM3*, *TPM4*, *PPF1B1*, *RANBP2* and *SEC31L1* genes in inflammatory myofibroblastic tumor (IMT).

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

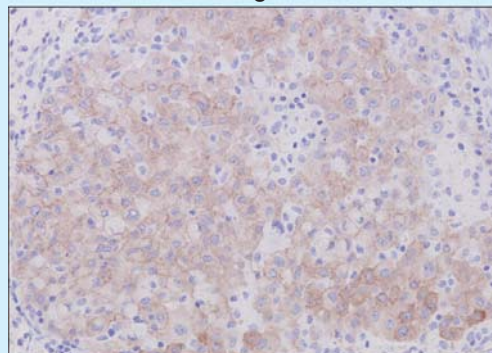
Recently, other *ALK* fusion genes with *EML4* gene, *KIF5B* gene or *KCL1* gene in non-small cell carcinoma of lung, *SEC31A* gene or *SQSTM1* gene in ALK-positive large B-cell lymphoma and *VCL* gene in renal cell cancer have been also reported.

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

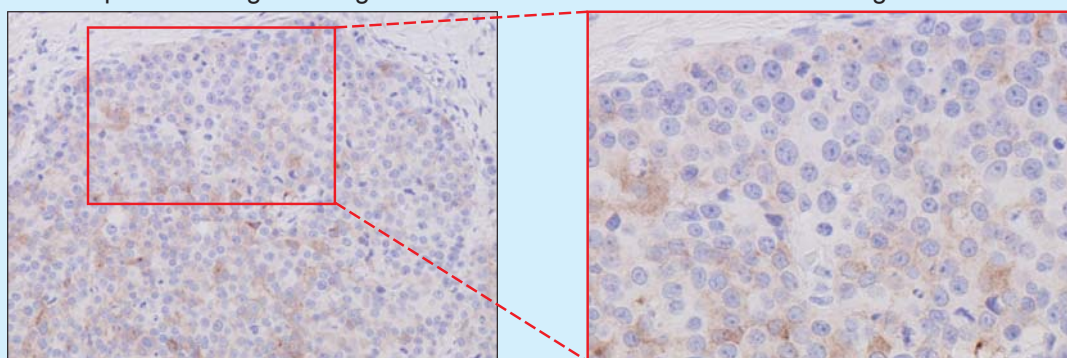
Product Name		Size	Code
N-Histofine® ALK Control Slides		5 slides	417081F

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 oncokinin 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, PPF1BP1-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



Distributed in North America by:
COSMO BIO USA
 2792 Loker Ave W, Suite 101
 Carlsbad, CA 92010
 TEL: 760-431-4600
 FAX: 760-431-4604
 email: info@cosmobiousa.com
 web: www.cosmobiousa.com

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>

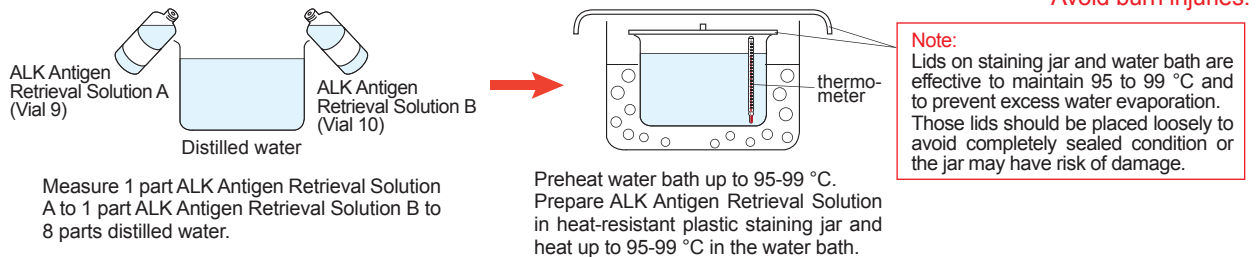
Staining Procedure

- 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.

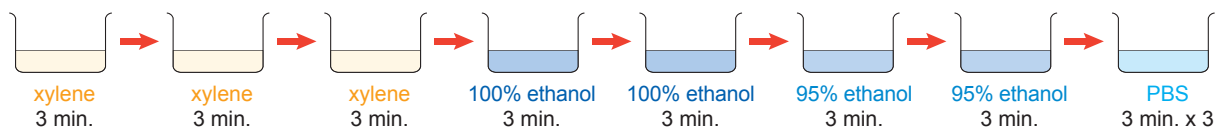
• Preparation of tissue sections



• Preparation of ALK Antigen Retrieval Solution



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

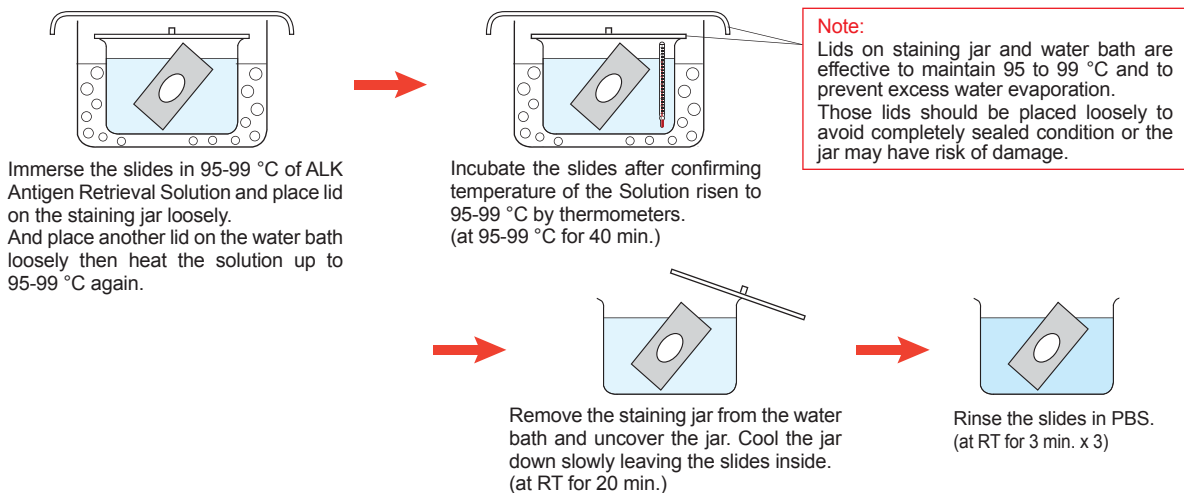


- Remove excess xylene and ethanol at each step.
- Change xylene and ethanol as appropriate to complete deparaffinization and rehydration.

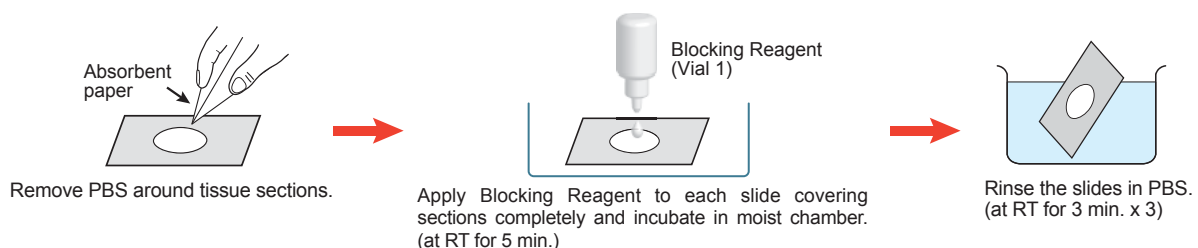
• Antigen Retrieval

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

Avoid burn injuries.

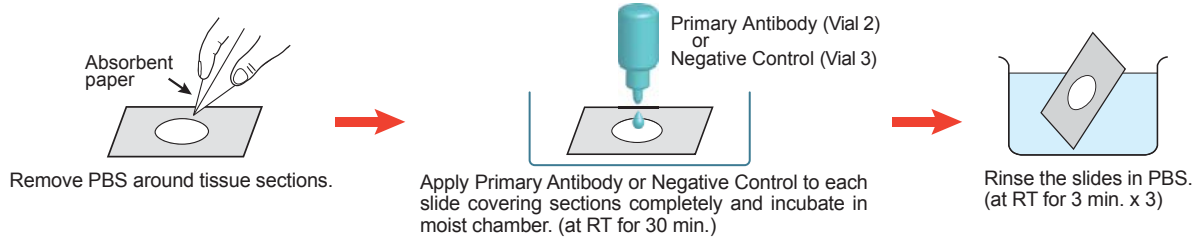


• Treatment with Blocking Reagent* (Removal of endogenous peroxidase) *3V/V% Hydrogen peroxide solution

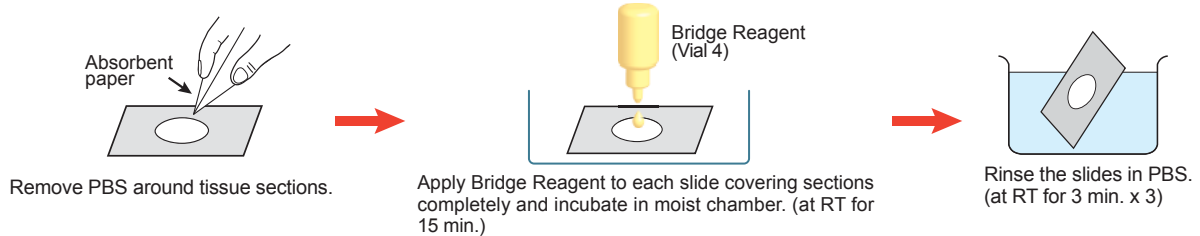


●Addition and reaction of Primary Antibody* or Negative Control**

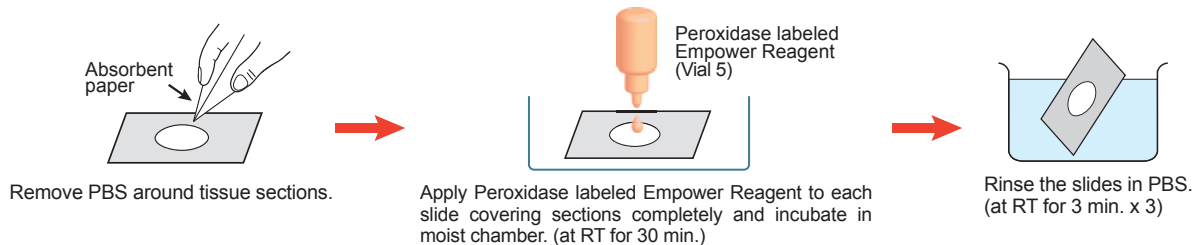
* anti-ALK mouse monoclonal antibody (5A4)
** Mouse IgG



●Addition and reaction of Bridge Reagent

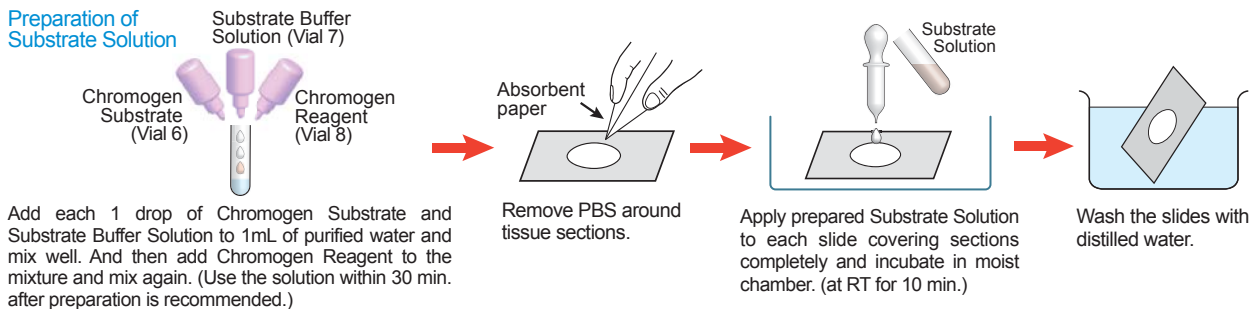


●Addition and reaction of Peroxidase labeled Empower Reagent



●Preparation, addition and reaction of Substrate Solution

Preparation 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.

N-Histofine® ALK Control Slides

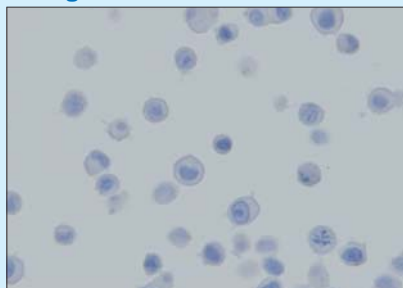
Code: 417081F Size: 5 slides

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.

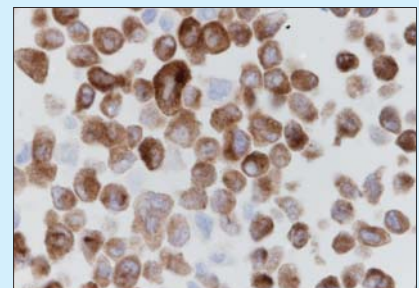
Both formalin fixed paraffin embedded cell lines of NCI-H2228 (Positive) and SK-BR-3 (Negative) are mounted on each slide.

■ 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 (+)