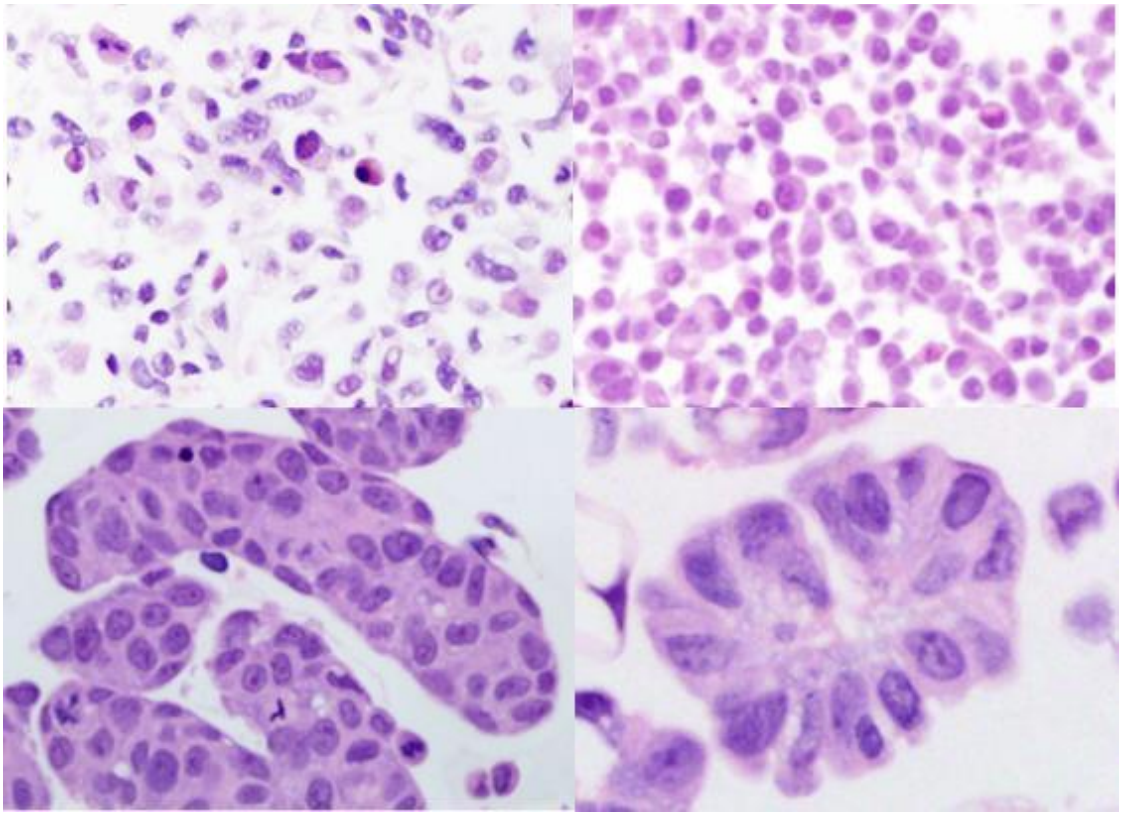
Interpretation Guide
Reference Range Cell Line

ALK-Lymphoma Cell Line Control (NPM-ALK)

Product Codes:
CS-NPMALK-2/5
SAM-CS-NPMALK-2/2
CBLK-NPMALK-2

StatLab Medical Products are proud to release a range of cost effective control slide products designed to help Pathologists and Histotechnologists maintain confidence in their IHC and ISH assays within their laboratory.



1. What is ALK?

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor encoded by the *ALK* gene. Synonyms include:

- CD246
- C2orf2
- EMAP-4
- ELP120
- NBLST3

Little is known of the specific function of ALK, however, it's understood to have a role in embryogenesis and the early development of the brain by regulating the proliferation of nerve cells.[1] in adults its expression is restricted to a few organs including brain, testis, small intestine, prostate and colon.[2]

2. Role of ALK in Cancer

The ALK gene is found on the short arm of chromosome 2. As an oncogene it was first identified as a translocation in anaplastic large cell lymphoma (ALCL) t (2;5)(p23;q35). In this instance the translocation caused a fusion product with the nucleophosmin gene: NPM-ALK.[2] In reality the ALK translocation is a promiscuous event and associated with numerous fusions in multiple malignancies, see tables 1 and 2.[3]

Table 1. Chromosomal translocation and fusion proteins in solid tumors involving ALK

Disease	Chromosomal rearrangement	Fusion protein	Frequency (%)
NSCLC	inv(2)(p21;p23)	EML4-ALK	2-5
	t(2;3)(p23;q21)	TFG-ALK	2
	t(2;10)(p23;p11)	KIF5B-ALK	<1
	t(2;14)(p23q32)	KLC1-ALK	<5
	t(2;9)(p23;q31)	PTPN3-ALK	ND
IMT	t(1;2)(q25;p23)	TPM3-ALK	0.5
	t(2;19)(p23;p13)	TPM4-ALK	<5
	t(2;17)(p23;q23)	CLTC-ALK	<5
	inv(2)(p23;q35)	ALK-ATIC	<5
	t(2;11;2)(p23;p15;q31)	CARS-ALK	<5
	t(2;2)(p23;q13)	RANBP2-ALK	<5
	inv(2)(p23;p15;q31)	RANBP2-ALK	<5
	t(2;4)(p23;q21)	SEC31L1-ALK	<5
BC	inv(2)(p21;p23)	EML4-ALK	<5
CRC	inv(2)(p21;p23)	EML4-ALK	<5
	t(2;2)(p23.3)	C2orf44-ALK	<5
ESCC	t(2;19)(p23;p13)	TPM4-ALK	ND
RCC	t(2;10)(p23;q22)	VCL-ALK	ND
	t(1;2)(q25;p23)	TPM3-ALK	ND
	inv(2)(p21;p23)	EML4-ALK	ND

NSCLC; non-small cell lung cancer. IMT; inflammatory myofibroblastic tumor, BC; breast cancer, CRC; colorectal cancer, ESCC; esophageal squamous cell carcinoma, RCC; renal cell carcinoma, ND; not determined.

Table 2. Chromosomal translocations and fusion proteins in hematologic malignancies involving ALK gene.

Disease	Chromosomal rearrangement	Fusion protein	Frequency (%)
ALCL	t(2;5)(p23;q35)	NPM-ALK	75-80
	t(2;17)(p23;q25)	ALO17-ALK	<1
	t(2;3)(p23;q21)	TFG-ALK	2
	t(2;X)(p32;q11-q12)	MSN-ALK	<1
	t(1;2)(q25;p23)	TPM3-ALK	Dec-18
	t(2;19)(p23;p13)	TPM4-ALK	<1
	inv(2)(p23;q35)	ATIC-ALK	2
	t(2;22)(p23;q11.2)	MYH9-ALK	<1
	t(2;17)(p23;q23)	CLTCL-ALK	2
DLBCL	t(2;5)(p23;q35)	NPM-ALK	ND
	t(2;17)(p23;q23)	CLTC1-ALK	ND
	t(2;5)(p23.1;q35.3)	SQSTM1-ALK	ND
	ins(4)(2;4)(p23;q21)	SQSTM1-ALK	ND
	t(2;4)(p24;q21)	SEC31A-ALK	ND
HL	t(2;5)(p23;q35)	NPM-ALK	ND

ALCL; anaplastic large cell lymphoma, DLBCL; diffuse large B cell lymphoma;HL: Hodgkin lymphoma; ND; not determined

Most recently, therapy for the fusion EML4-ALK in lung cancer has created fresh focus on the detection of ALK but in relation to non-small cell lung cancer (NSCLC)[3,4,5] rather than NPM-ALK, which has long been used in the diagnosis of ALCL.

3. Cell Line Controls

This product is sold in two formats. Pre-prepared slides: ***CS-NPMALK-2/5***, as in figure 2 below.

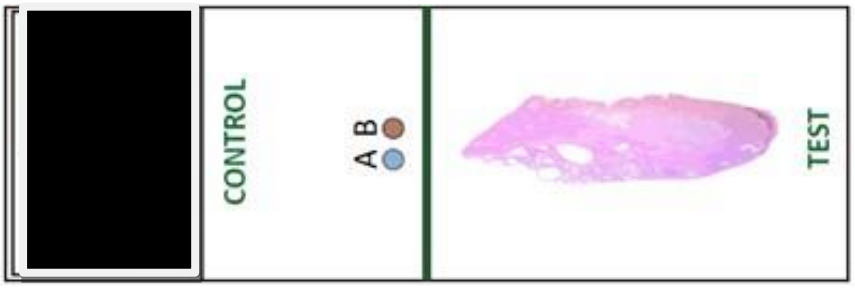


Figure 2: CS-NPMALK-2/5 Cell Line Control Slide(s)

Or in a cell microarray (CMA) paraffin wax block: ***CBL-NPMALK-2/5***, as illustrated in figure 3.

Figure 3: CBL-NPMALK-2 TMA block

Both formats have their merit depending on the needs of the laboratory. The slides offer ease of use and save time in preparation. However, in high volume centers the blocks provide a more cost effective solution and fit into the work flow of the laboratory easily.

In either case the analyte controls demonstrate that the reagents employed to perform the assay have worked effectively in combination with the staining protocol. They determine:

- Reagent performance
- Correct implementation of the staining protocol (manual or automated)

They confer confidence i.e. those reviewing the slide can be reassured that if the control has worked appropriately, the assay has worked.

The expression patterns of the 2 cell lines contained within ***CS-NPMALK-/52*** and ***CBL-NPMALK-2*** are shown below, table 3:

Cell Lines	ALK Gene Status	ALK Lymphoma
A	Negative	Negative
B	ALK translocation positive	Positive for NPM-ALK

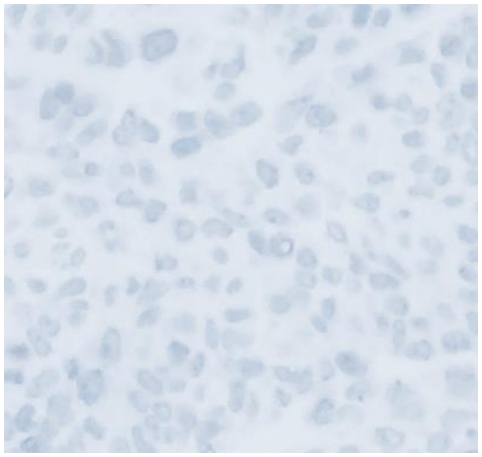
Table 3. Cell status for ALK-Lymphoma

4. Expected Staining Results for ALK-Lung

The following section gives micrographs of the expected results obtained with each of the cells assessed by IHC with Confirm Anti-ALK (ALK01) primary antibody from Roche/Ventana. For more information please contact ihctech@statlab.com.

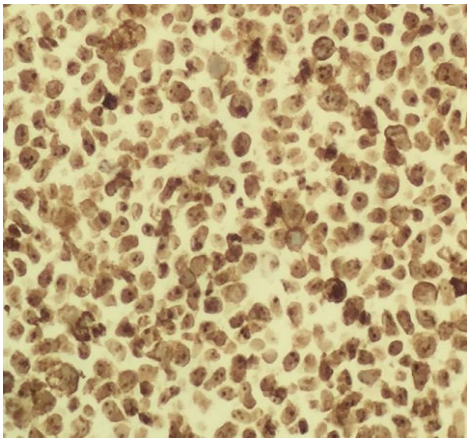
4.1 ALK-Lymphoma harboring ALK-NPM fusion protein. Immunohistochemistry: Roche/Ventana, anti-ALK (ALK01) Mouse Monoclonal Primary Antibody. (790-2918)

A



No staining present.

B



>95% of cells demonstrate intense cytoplasmic staining.

5. References

1) Roskoski R Jr. Anaplastic lymphoma kinase (ALK): structure, oncogenic activation, and pharmacological inhibition. Pharmacol Res. 2013 Feb;68(1):68-94.
2) Morris SW, Kirstein MN, Valentine MB, Dittmer K, Shapiro DN, Look AT, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994;263:1281-4
3) Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, Liu D. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015 Feb 27;8(1):17
4) Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D,Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA.Crizotinib versus chemotherapy in advanced ALK-positive lung cancer.N Engl J Med. 2013 Jun 20;368(25):2385-94.
5) Sasaki T, Rodig SJ, Chirieac LR, Jänne PA. The Biology and Treatment of EML4-ALK Non-Small Cell Lung Cancer. Eur J Cancer. 2010 July ; 46(10): 1773–1780.
6) UKNEQAS Journal: Immunocytochemistry. Run 108/37. Assessments Dates: 5th-23rd January 2015.http://www.ukneqasicc.ucl.ac.uk/run_108_journal.pdf
7) NordiQC Lung Anaplastic Lymphoma Kinase (lu-ALK) Assessment Run 39 2013 http://www.nordiqc.org/Run-39-B16-H4/Assessment/Run39_ALK.pdf
8) VENTANA ALK Scoring Interpretation Guide for non-small cell lung carcinoma (NSCLC). 1011879EN, October 2012, Revision D.

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