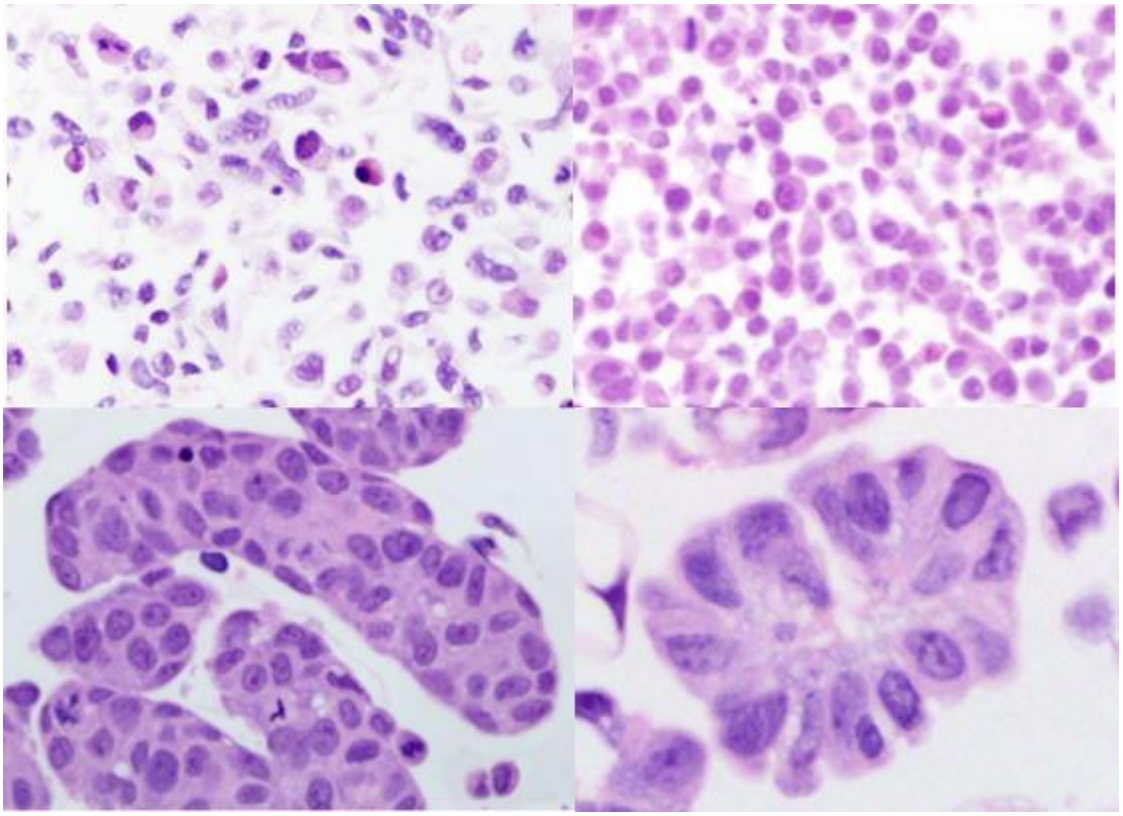
Interpretation Guide
Reference Range Cell Line

ALK-Lung Cell Line Control
(EML4-ALK)

Product Codes:
CS-EML4-2/2
SAM-CS-EML4-2/2
CBLK-EML4-2/5

Contents

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. Detecting EML4-ALK

Until recently, ALK translocations in NSCLC have been detected by either polymerase chain reaction (PCR) or visually assessed by fluorescence in situ hybridization (FISH). For some time it was believed that immunohistochemistry (IHC) was not sensitive enough for the detection of EML4-ALK. However, with the re-optimization of some of the currently available antibodies and the advent of new clones and detection systems, IHC is becoming more widely adopted.

The most commonly used antibodies on the market, clones 5A4, D5F3 and ALK1,[6] all recognize C-terminus (see respective vendor data sheets), the green sections in Figure 1 below. This is the conserved ALK region harboring the tyrosine kinase domain. Therefore, all of these antibodies should recognize the ALK fusion proteins. In practice the efficacy of the antibody or its affinity for the target epitope, the relative availability of fusion protein and appropriate epitope retrieval and IHC protocols mean that variation is seen from laboratory to laboratory. This is evidenced in external quality assurance programs.[6,7]

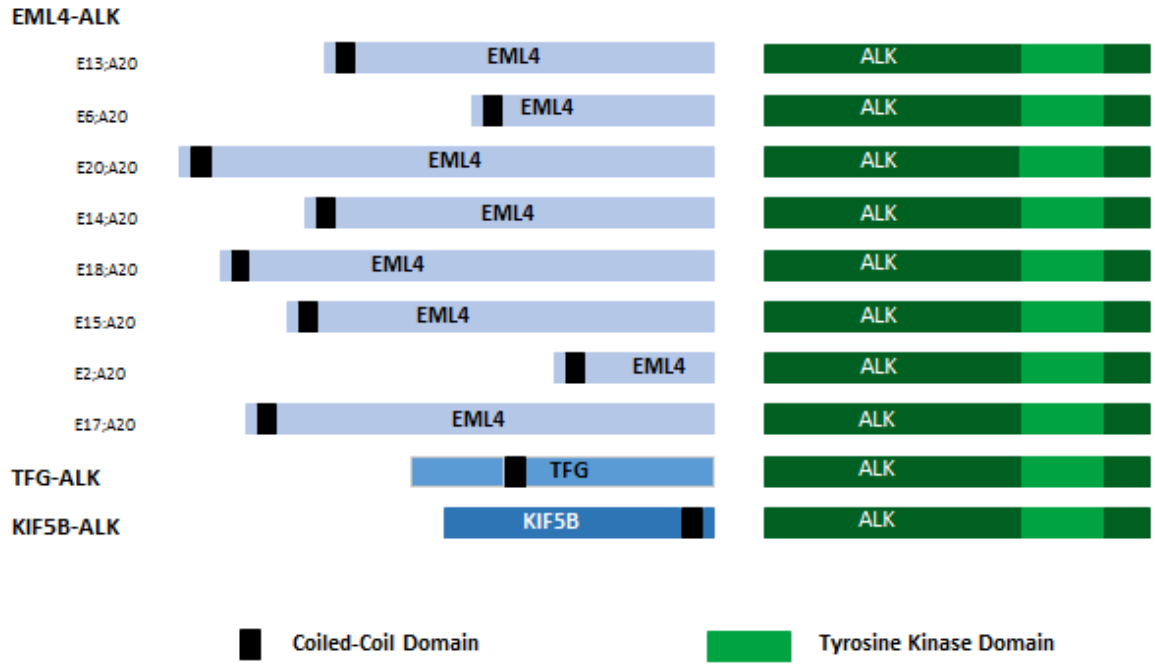


Figure 1. Different variants of EML4-ALK and non-EML4 fusion partners. The nomenclature refers to the exon in EML4 translocated to the exon in ALK (adapted from reference 5).

4. Cell Line Controls

This product is sold in two formats. Pre-prepared slides: ***CS-EML4-2/5***, as in figure 2 below.

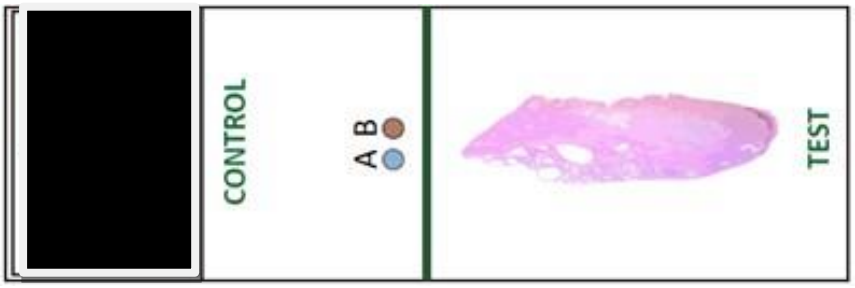


Figure 2: Cell Line Control Slide

Or in a cell microarray (CMA) paraffin wax block: ***CBL-EML4-2***, as illustrated in figure 3.

Figure 3: CBL-EML4-2 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-EML4-2/5*** and ***CBL-EML4-2*** are shown below, table 3:

Cell Lines	ALK Gene Status	ALK Fusion Protein Expression
A	Negative	Negative
B	ALK translocation positive	Positive for EML4-ALK

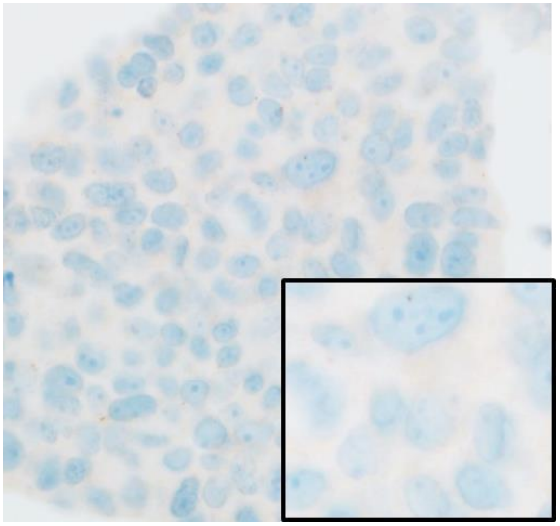
Table 3. Cell status for ALK-Lung

5. 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 the standardized companion diagnostic from Roche/Ventana and FISH assay from Abbott Molecular.

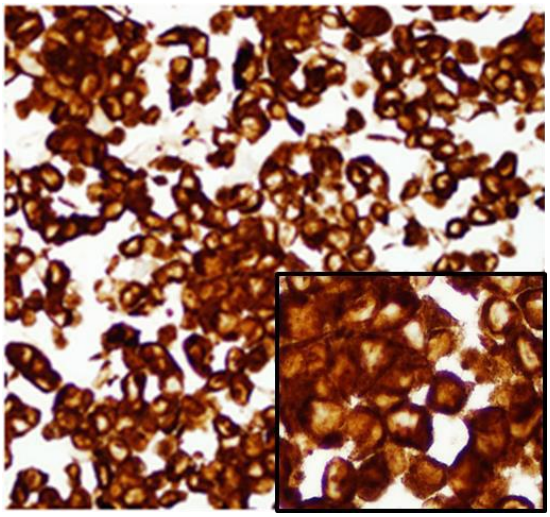
5.1 ALK Immunohistochemistry: Roche/Ventana, anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody. (790-4843)

A



No staining present.

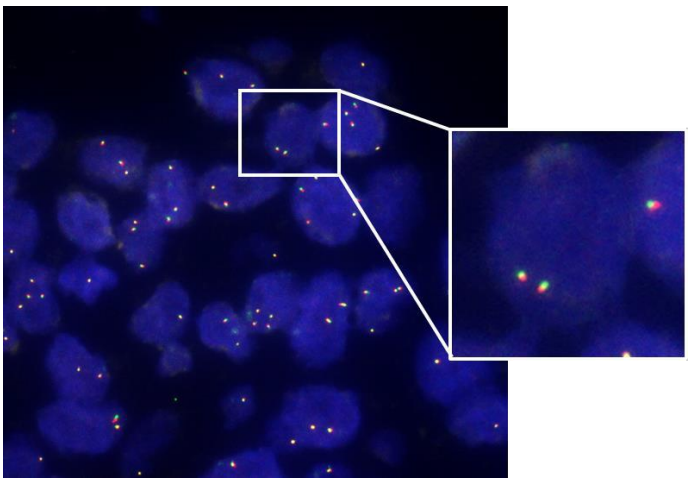
B



>95% of cells demonstrate intense cytoplasmic staining.

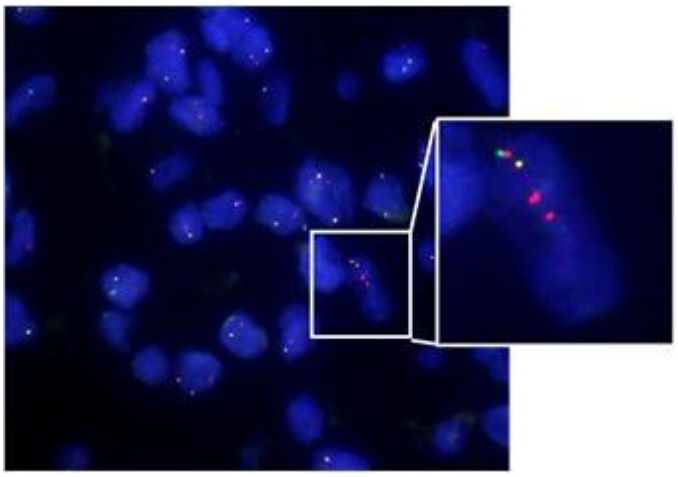
5.2 ALK translocation Fluorescence in situ hybridization: Abbott Molecular, Vysis ALK Break Apart FISH Probe Kit. 06N38-020.

A



Signals overlapped or clustered together <2 signals apart.

B



Green and red signals clearly split. >2 signal diameters apart of one another.

6. Troubleshooting

The Roche/Ventana assay is known to produce non-specific cytoplasmic punctate staining. It is documented in the VENTANA ALK Scoring Interpretation Guide for non-small cell lung carcinoma (NSCLC).[8] these observations have been seen in the negative cell line in the course of assessment. Its occurrence is erratic and due to the amplification steps associated with the detection system Optiview™. The Interpretation guide from Roche/Ventana is clear that weak diffuse cytoplasmic staining should be considered negative. Figure 4 below shows an example of this in a negative cell sample.

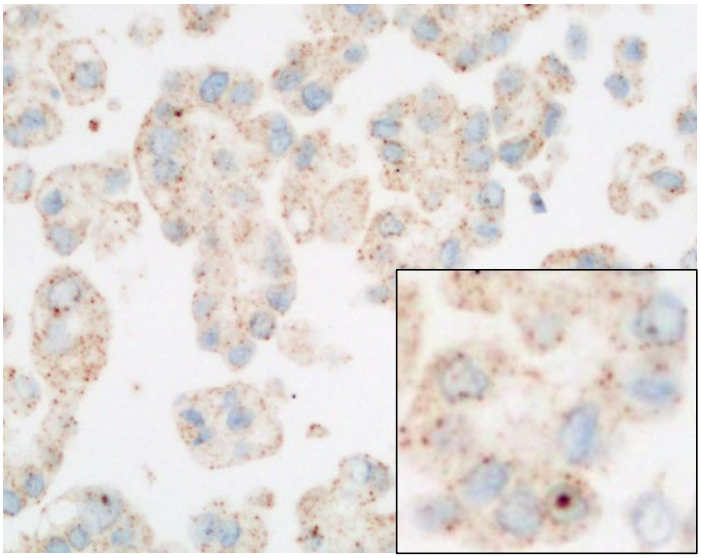


Figure 4. Non-specific cytoplasmic staining, note the larger deposits (bottom of insert). This should be considered negative.

7. References

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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.
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8) VENTANA ALK Scoring Interpretation Guide for non-small cell lung carcinoma (NSCLC). 1011879EN, October 2012, Revision D.

For more information, contact ihctech@statlab.com. or visit our website www.StatLab.com and download our Interpretation Guide.