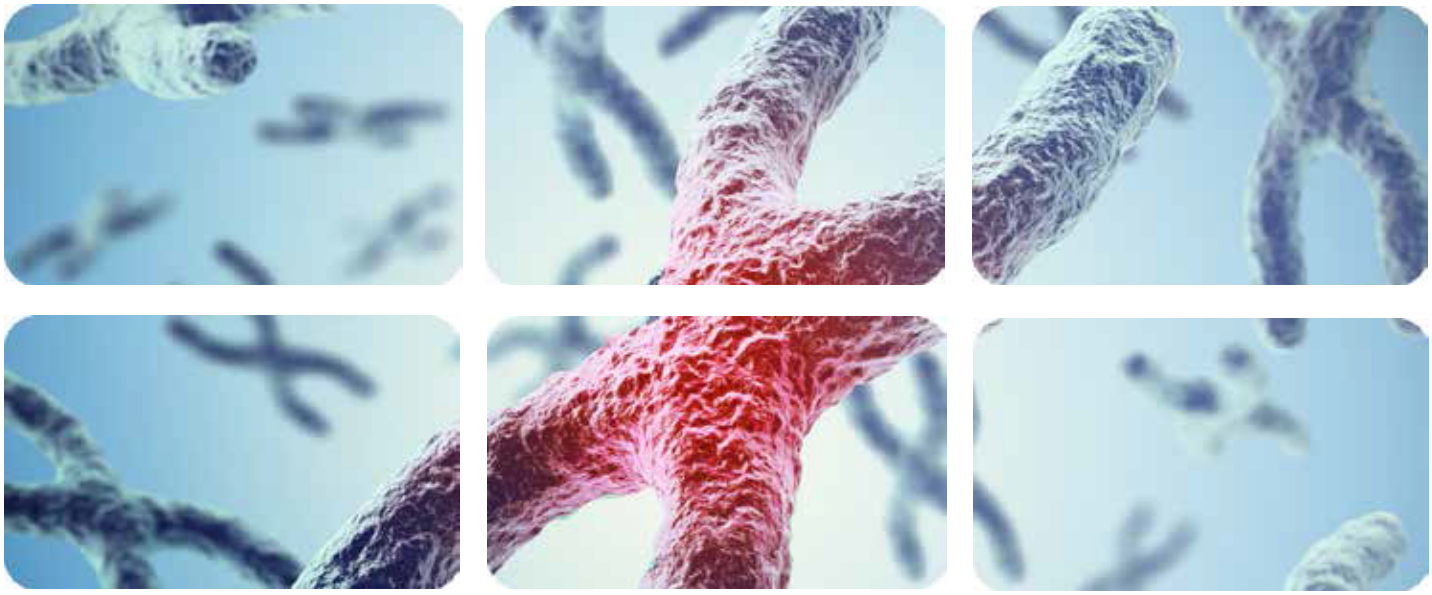
DIACARTA™

QFusion™

Improves the quality and productivity of EML4-ALK and KIF5B-ALK fusion gene detection



Introduction

EML4-ALK has been emerged as one of the most important driver oncogenes in lung cancer and the first targeted fusion onco-kinase to be identified in 4-6% of lung adenocarcinomas. Other non-EML4 fusion partner genes including KIF5B, TFG, and KLC1 have also been reported. Over the last few years, ALK inhibitors have shown significant benefits in the management of ALK-positive non-small cell lung cancer (NSCLC) compared to conventional chemotherapy.

QFusion™ fusion gene detection kit provides an easy-to-use multiplex RT-qPCR assay that simultaneously detect the most prevalent EML4-ALK and KIF5B gene fusion breakpoints. This highly specific and sensitive assay requires a minimal amount of RNA, and it is a rapid and reliable alternative to the laborious FISH test.

ALK Fusion Variants Detected by QFusion™

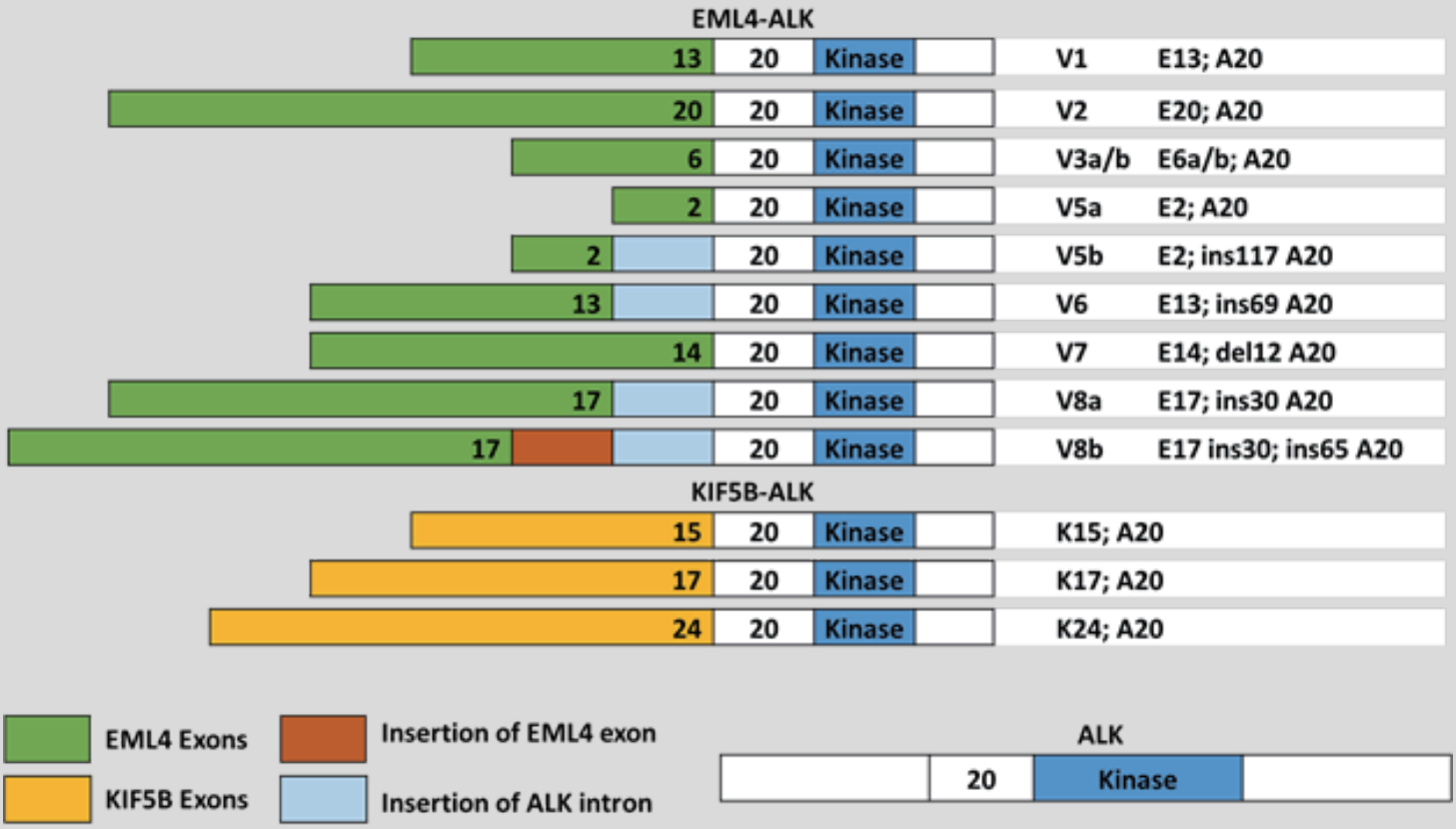


Figure 1. Schematic that shows the ten EML4-ALK and three KIF5B-ALK fusion gene variants detected by the QFusion™ assay.

QFusion™ Assay Strategy

To reduce both the material input and operational time, DiaCarta has designed and developed a multiplex real-time RT-qPCR assay to detect as many ALK fusion variants in a **single-tube** assay. It is a **one-step** assay – the target RNAs will be reverse transcribed and the resulting cDNAs will be amplified by the qPCR containing pairs of primers that span the junctions of the chimeric cDNA variants as well as a pair of primers for an internal control cDNA. It also contains dual-labeled hydrolysis probes (Taqman®) for both the fusion and internal control genes.

Tired of FISH Method?

Table 1. ALK fusion variant detection by the qPCR approach provides multiple advantages when comparing to the fluorescence in situ hybridization (FISH) method.

	qPCR	FISH
Comprehensive Variants	✓	✗
Low Sample Input	✓	✗
Quick Results	✓	✗
Objective Interpretations	✓	✗
Multiplex Reactions	✓	✗

UNIQUE

Only commercial qPCR kit for EML4-ALK and KIF5B-ALK fusion variants

COMPREHENSIVE

Cover 95% EML4-ALK and 90% KIF5B-ALK variants in COSMIC database

EASY TO OPERATE

One-tube multiplex qPCR reactions and only 50 ng RNA as assay input

SPECIFIC & SENSITIVE

No cross reactivity with wild-type RNA and 50 fusion templates detected per reaction

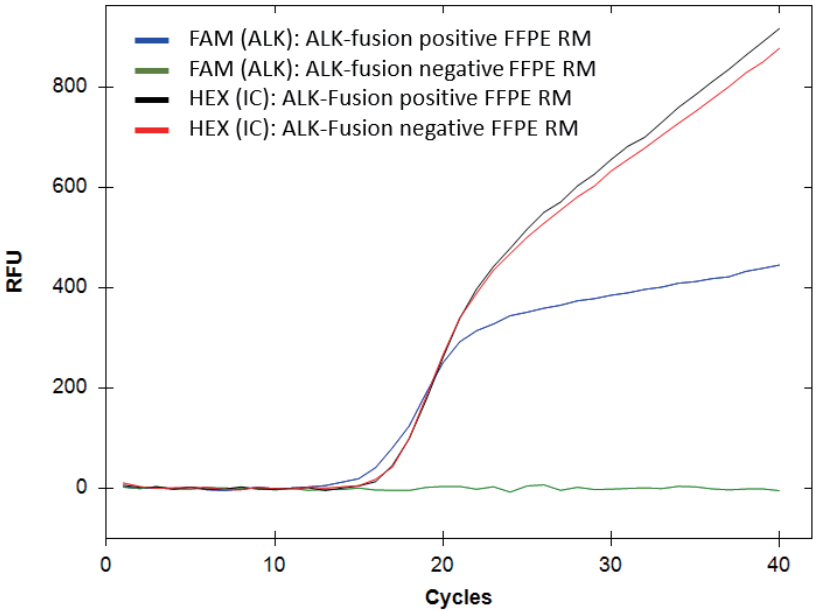
RAPID

Total turnaround time approx. 1.5 hours

VERSATILE

Assay validated in wildly used real-time qPCR machines

Data



EML4-ALK fusion gene variant 1 can be successfully detected from the ALK fusion positive FFPE reference material (RM) (blue line) but not the negative RM (green line). The internal control (IC) assay similarly amplifies the positive and negative FFPE RMs (black and red lines), indicating that the total RNA input extracted from these RMs (50 ng) is about the same.

Figure 2. qPCR amplification curves generated by QFusion™ ALK fusion gene assays for FFPE reference materials (RMs).

Product Specifications

Fusion Variants	EML4-ALK: V1, V2, V3a/b, V5a/b, V6,V7, V8a/b KIF5B-ALK: (K15; A20), (K17; A20), (K24; A20)
Sample	FFPE tissues from non-small cell lung cancer (minimum 50 ng RNA)
Pack Size	Reagents sufficient for analyzing 20 samples per kit
Instruments	Roche LC 96, LC 480 II, and Bio-Rad CFX384
Detection Channels	FAM, HEX
Turnaround Time	qPCR setup: ~0.5 hour; assay run time: ~ 1 hour
Stability	Stable for 12 months at -25ºC to -15ºC

Ordering Information

Product Name	QFusion™ EML4-ALK and KIF5B-ALK Fusion Gene Detection Kit (20 Samples)
Catalog Number	DC-20-0020R