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# Product Manual

# Of

# EML4-ALK Fusion Gene Detection Kit

## For simultaneous detection of 15 fusion variants in one real-time PCR reaction

*Note: Research Use Only. Not intended for use in diagnostic procedures.*

For the following products:

Cat. No. 1-200-10010
Cat. No. 1-200-10020

## PURPOSE OF THE TEST

The EML4-ALK Fusion Gene Detection Kit is a novel, multiplex reverse transcription real-time PCR (RT-qPCR) system for the simultaneous detection of 15 fusion variants, as shown in Table 1. Fusion gene and control gene transcripts are co-amplified in each reaction, and identified by specific fluorescent probes. Rapid results can be obtained within 2-3 hours. The screening system provides a sensitive, specific, reliable and cost-effective tool that is easy to perform for the routine detection of EML4-ALK fusion transcripts.

| Fusion Variants | EML4 Spliced Exon | ALK Spliced Exon |
|---|---|---|
| EML4-ALK V1 | 13 | 20 |
| EML4-ALK V2 | 20 | 20 |
| EML4-ALK V3a/b/c | 6 | 20 |
| EML4-ALK V4 | 14 | 20 |
| EML4-ALK V5a/b | 2 | 20 |
| EML4-ALK V6a/b | 13 | 20 |
| EML4-ALK V7 | 14 | 20 |
| EML4-ALK V8a/b/d | 17 | 20 |
| EML4-ALK V10 | 18 | 20 |

Table1: Fusion Variants detected by EML4-ALK kit.

## PRINCIPLE OF THE TEST

The EML4-ALK Fusion Gene Detection Kit is a RT-qPCR based assay for the detection of EML4-ALK associated fusion gene transcripts found in total RNA from lung cancer tissues. Ready to use cDNA synthesis reagents and qPCR primer/probe mixes are included in the kit. An example of the primer/probe design is shown in Figure 1.

The EML4-ALK Fusion Gene Detection was developed based upon the platform of the Yin-Yang Probe, a novel technology for nucleic acid detection and related applications (Patent No. US7799522). The probe itself is non-fluorescent due to the close proximity of the fluorophore and quencher. When the Yin-Yang probe is in the presence of its target DNA, the target strand displaces the negative strand along with its quencher, and the fluorophore becomes fluorescent. This process is shown in Figure 2.

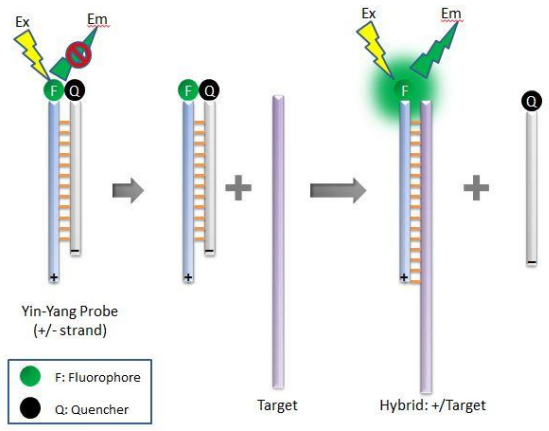


Figure 2: Principle of Ying-Yang Probes.

In the process of qPCR, the positive probes bind to the PCR products during the annealing step, and release fluorescence signals. Signals are collected in the annealing step.

cDNA is synthesized by adding purified RNA to the ready to use cDNA reaction mix. The resulting cDNA is mixed with the specific PCR primers and probes mix for detection of fusion variants. qPCR is performed in a real-time PCR instrument with optical filters for the detection of FAM and HEX.

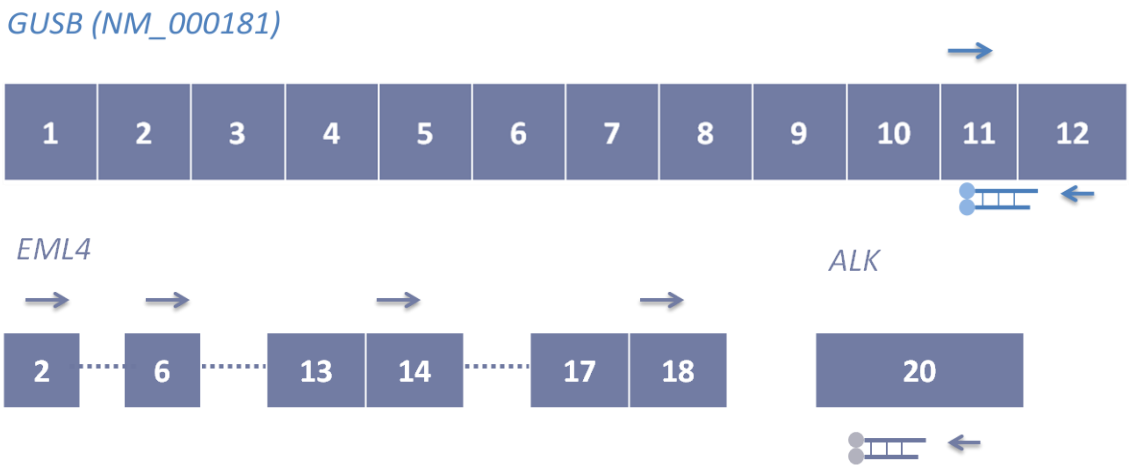


Figure 1: Schematic of the primers and probes designed in the EML4-ALK detection kit. Graph on the top shows an example of the GUSB (internal control). Graph on the bottom shows the primer/probe design to detect EML4-ALK variants. Primers are depicted in horizontal blue arrows.

## REQUIRED EQUIPMENT

- Table centrifuge
- Thermal Heating Block
- qPCR instrument with filters for FAM (Abs 495 nm, Em 520 nm) and HEX (Abs 535nm, Em 556 nm)
- Pipettes and sterile RNase free filter tips

## KIT COMPONENTS AND STORAGE

The kit contains sufficient reagents to carry out 10 or 20 tests depending on the size (Table 2). The kit must be stored at -20°C away from light. Avoid frequent/unnecessary freeze-thaw cycles of the kit.

Table2: Kit Components

| | | 10-test kits | 20-test kits |
|---|---|---|---|
| REVERSE TRANSCRIPTION REAGENTS | RT buffer | 70 µL | 140 µL |
| | Reverse Transcriptase | 14 µL | 28 µL |
| PCR REAGENTS | 4X Primer/Probe mix | 70 µL | 140 µL |
| | 2x QD Fast Supermix | 125 µL | 250 µL |
| CONTROLS | Positive Control Plasmids | 25 µL | 50 µL |
| | Water | 500 µL | 1 mL |

## PRECAUTIONS

Due to the inherent instability of RNA, fresh patient samples or samples stored at -80 °C are preferred. When working with RNA, always wear gloves to avoid RNAse from the hands. Extracted RNA should be treated for cDNA synthesis, or stored at -80 °C for no more than 3 months.

The use of aerosol barrier pipette tips is highly recommended during the entire procedure.

## PROCEDURE

### *RNA preparation*

Extracted RNA should be analyzed for concentration and quality. $A_{260}/A_{280}$ values should be between 1.9 and 2.1 while $A_{260}/A_{230}$ values should be greater than 2.0.
Total RNA can be stored at -80°C before use in cDNA synthesis.

### *cDNA synthesis*

- Prepare the 20 µL RT reaction mix on ice as shown in Table 3. Total RNA amount should be 0.1 µg to 1 µg in the 20 µL reverse transcription reaction. Incubate reaction mix at 25 °C for 5 minutes.
- Incubate the cDNA synthesis reaction at 37 °C for 30 minutes.
- Inactivate reverse transcriptase enzyme by heating reaction mix at 85 °C for 5 minutes.

- Dilute each of the cDNA sample tubes with 30 µL dd$H_2O$. 5 µL cDNA sample will be used for the following PCR reaction. The leftover cDNA can be stored for repeating tests.

Table3: cDNA synthesis reaction mix

| Reagents | Volume(µL) per 20 µL reaction |
|---|---|
| RT buffer | 4.8 |
| RT Enzyme | 1 |
| Total RNA | x |
| DEPC $H_2O$ | 14.2-x |

### *Real-time qPCR*

- Make the PCR master mix: If the number of tests that will be performed is n, the volume the master mix needs to be n* 10 µL **2X QD Fast Supermix** + n* 5 µL **4X Primer/Probe mix**.
- Pipette 15 µL of the master mix into each well.
- For experimental samples add 5 µL cDNA sample to each well. For positive control samples, add 5 µL of positive control plasmid to each well. For negative control samples, add 5 µL of provided nuclease free $H_2O$ to each well.
- Run the qPCR reaction with the following program, collect data at the 60℃ annealing step :

| | Thermal cycle | Cycle # |
|---|---|---|
| Preheating | 95℃ 3 min | 1 |
| PCR Cycle | 95℃ 20 sec | 45 |
| | 60℃ 45 sec (detection channels: FAM and HEX) | |

## Data Analysis and Interpretation

After the run is finished, the threshold line should be carefully set up to allow accurate $C_t$ determination. $C_t$ values below 10 are not valid.

All reactions should give FAM signal for the Internal Control and the $C_t$ values should be less than 35. $C_t$ values greater than 35 for the internal control means the sample concentration is too low or the PCR reaction has been inhibited for false negative results.

The $C_t$ values should be below 30 for the positive control in the HEX channel. For fusion sample analysis, $C_t$ values for RNA sample below 35 are determined as positive. $C_t$ values above 35 might be non-specific amplification. Repeat the test with fresh RNA, if the second test is positive, the sample is positive for the corresponding fusion gene. Other diagnostic techniques are highly recommended to confirm the results from positive tests with $C_t$ values above 35. Interpretation table is shown in Table 4.

Table4: Data interpretation

| Fluorescence Signal | Results |
|---|---|
| **FAM+HEX** | **EML4-ALK Positive** |
| **FAM Only** | **EML4-ALK Negative** |

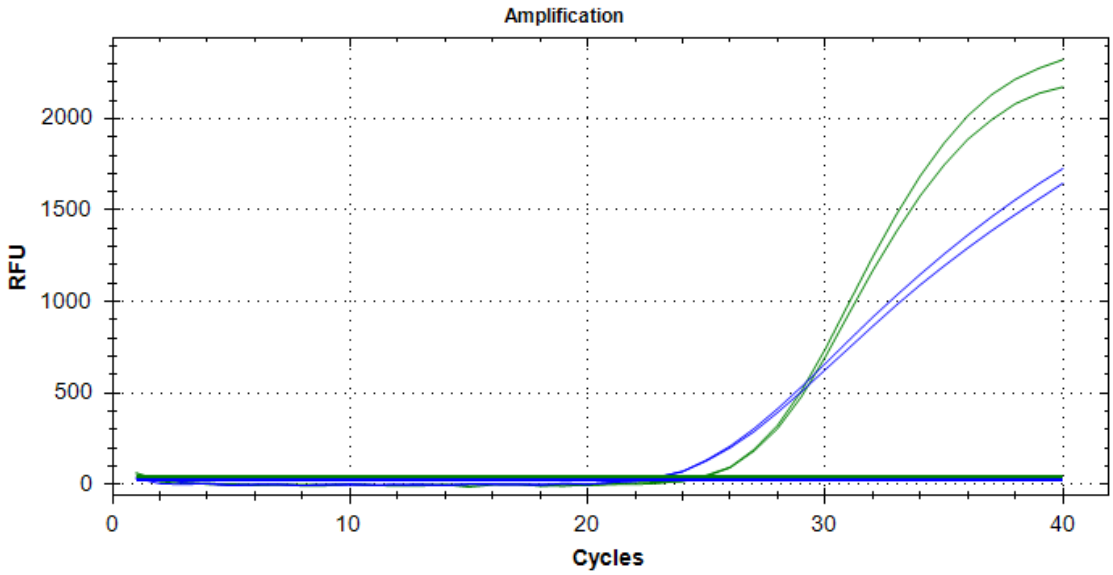


Figure 3: Example of the amplification curve of EML4-ALK positive sample. The internal control is shown in green and the positive control is shown is blue.