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Product Manual

Of

Lung Cancer ALK/ROS1/RET Testing Panel

For simultaneous detection of six lung cancer fusion genes in one real-time PCR reaction

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

For the following products:

Cat. No. 1-200-20010
Cat. No. 1-200-20020

Contents

PURPOSE OF THE TEST

The Lung Cancer ALK/ROS1/RET Testing Panel is a novel, multiplex reverse transcription real-time PCR (RT-qPCR) system for the simultaneous detection of six fusion genes covering 35 fusion variants. Sample fusion gene and control gene transcripts are co-amplified in four reactions, and identified by specific fluorescent probes in each reaction. Rapid results can be obtained within 2-3 hours. This screening system provides a sensitive, specific, reliable and cost-effective tool that makes it easy to perform routine screenings of lung cancer fusion transcripts.

PRINCIPLE OF THE TEST

The Lung Cancer ALK/ROS1/RET Testing Panel is a RT-qPCR based assay for the detection of lung cancer fusion gene transcripts found in total RNA. Ready to use cDNA synthesis reagents and qPCR primer/probe mixes are included in the kit.

cDNA is synthesized by adding purified RNA to the ready to use cDNA reaction mix. The resulting cDNA is mixed with the appropriate specific PCR primers and probes mixes (as labeled A~D) for the detection of fusion genes. Four reactions are required for the screening of each cDNA sample. Real-time PCR is performed on instrument with optical filters for the detection of FAM, HEX and Cy5. Real-time PCR amplification plots and C_t (threshold cycle) values are used for identification of the translocation and fusion gene transcript using a simple interpretation table.

The Lung Cancer ALK/ROS1/RET Testing Panel 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 below in Figure 1.

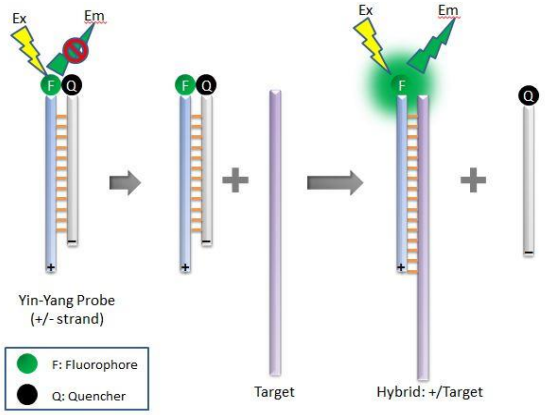

Figure 1: Principle of Ying-Yang Probes.

In the process of real-time PCR, the positive strands bind to the PCR products during the annealing step, and release fluorescence signals. Signals are collected at the annealing step. An example of the primer/probe design is shown below in Figure 2.

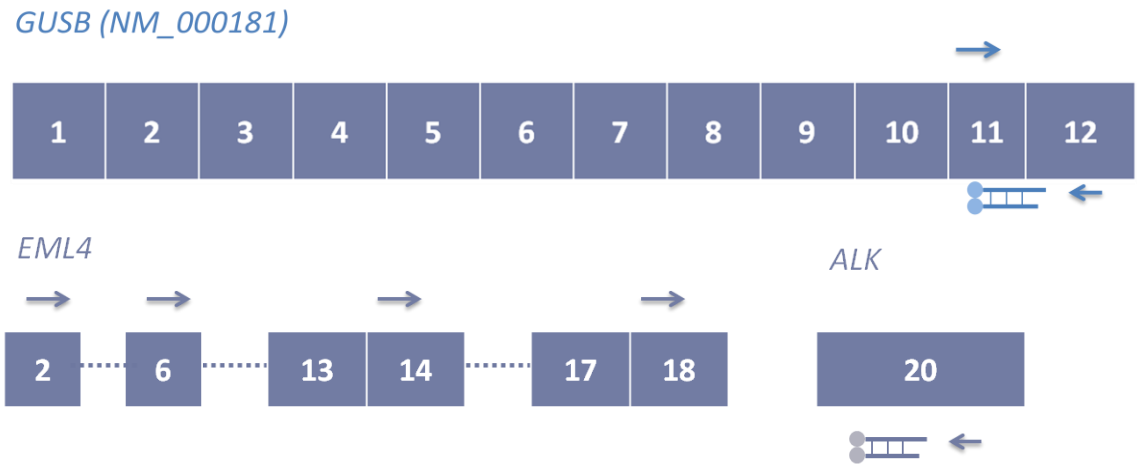

Figure 2: Schematic of the primers and probes designed in the Q-fusion screening kit. Graph on the top shows an example of GUSB (internal control). Graph on the bottom shows the detection of EML4-ALK.

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.

Laboratory workbenches and pipettes should be cleaned with bleach on a regularly scheduled basis.

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

REQUIRED EQUIPMENT

- Table centrifuge
- Thermal Heating Block
- qPCR instrument with filters for FAM (Abs 495 nm, Em 520 nm), HEX (Abs 535nm, Em 556 nm) and Cy5 (Abs 650 nm, Em 670 nm).
- Pipettes and sterile RNase free filter tips.

KIT COMPONENTS AND STORAGE

This kit contains sufficient reagents to carry out 10 or 20 tests depending on the size (Table 1). The kit must be stored at -20°C and away from light. Avoid frequent/unnecessary freeze-thaw cycles of the kit.

Table1: 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 A/B/C/D	4X 70 µL	4X 140 µL
	2x QD Fast Supermix	500 µL	1 mL
CONTROLS	Positive Control Plasmids	25 µL	50 µL
	water	500 µL	1 mL

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 2. Total RNA amount should be 0.1 µg to 1 µg in the 20 µL reverse transcription reaction.

Table2: 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

- Incubate reaction mix at 25 °C for 5 minutes.
- Incubate reaction mix 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 ddH_2O. Every cDNA sample will be added into 4 reaction tubes with 5 µL each for the simultaneous fusion gene screening test. The leftover cDNA can be stored for repeating test.

Real-time qPCR

- Make 4 PCR master mixes, one for each primer/probe mix labelled A, B, C or D: If the number of tests that will be performed is n, the volume of each of the 4 PCR master mixes needs to be n*10 µL 2X **QD Fast Supermix** + n* 5 µL 4X **Primer/Probe Mix**. For every sample, pipette 15 µL of one of the four PCR master mixes A, B, C, or D to each reaction.
- In a 96-well plate, add 15 µL of each master mix to each well. For experimental samples, add 5 µL of cDNA 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.
- Data collection is done during the 60°C annealing step.
- Run the qPCR reaction with the following program:

	Thermal cycle	Cycle #
Preheating	95°C 3 min	1
PCR Cycle	95°C 20 sec	45
	60°C 45 sec (detection channels: FAM, HEX and Cy5)	

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.

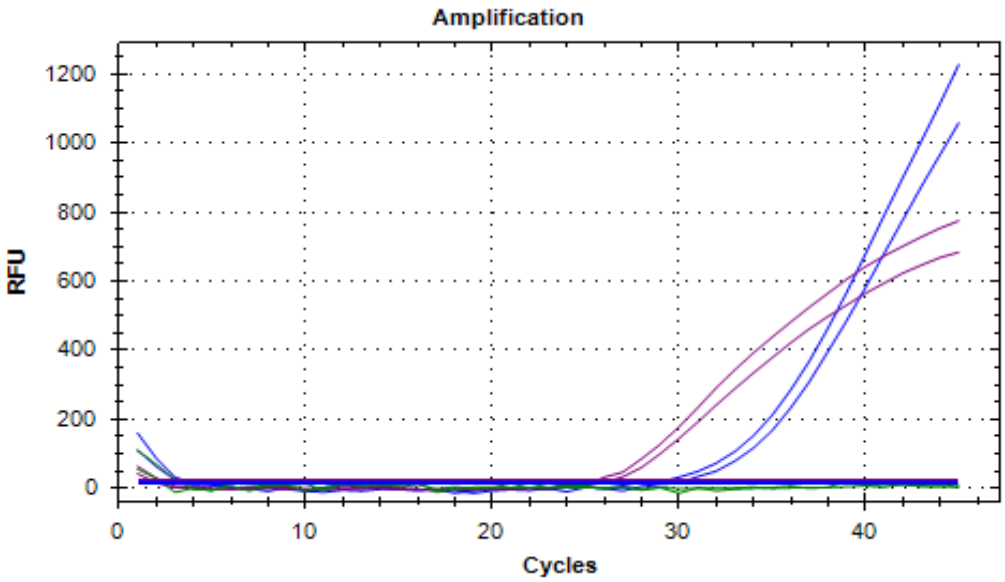

Figure 3: Example of the amplification curve for EML4-ALK positive sample. The internal control is shown in purple and the fusion type signal is in blue.

INTERPRETATION TABLE

TUBE	FLUORESCENCE SIGNAL	FUSION GENE
A	FAM	KIF5B-RET
	HEX	KIF5B-ALK
	Cy5	Internal Control
B	FAM	CD74-ROS1
	HEX	TFG-ALK
	Cy5	Internal Control
C	FAM	SLC34A2-ROS1
	HEX	EML4-ALK *1
	Cy5	Internal Control
D	HEX	EML4-ALK *2
	Cy5	Internal Control

*1 EML4-ALK fusion variants: V1, V2, V3a/b/c, V4, V5a/b, V6a/b, V7, V8a/b/d, V10

*2 EML4-ALK fusion variants: V8c, V9