EML4/ALK(V1,V2,V3A/B) REAL TIME PCR DETECTION KIT

ONKOTEST R6010-20

onkotest

Product Information

2-7% of patients with non-small cell lung cancer (NSCLC) have a rearrangement of the EML4 and ALK genes. Both genes are located on the short arm of chromosome 2. To date there are 15 different fusion transcript variants identified. The V1, V2 and V3a/b variants make up 72-80% of all EML4/ALK chimeric transcripts. The onkotest R6010-20 kit is able to detect these three variants with high sensitivity and specifity. The onkotest R6010-20 kit dose not detect other variants.

Kit Contents

Tube No.	Labelling and Contents	Volume
1	PCR Master Mix (5X)	220 µl
2	EML4-ALK primer mix	30 µl
3	Positive Control cDNA	10 µl
4	ß-actin Primer Mix	30 µl
5	dH_2O (PCR grade)	500 µl

Onkotest R6010-20 kit is designed to work with all Real time PCR devices.

Kit Description

Onkotest R6010-20, Complementer DNA (cDNA) converted from total RNA of the patients is used as template in the reactions. cDNA quality is checked by using PCR primers of an internal control gene (ß-aktin), provided with the kit. (This kit not included cDNA synthesis materials).
Real time PCR will amplify target the EML4/ALK (v1,v2,v3a/b) and Beta Actin regions by using highly specific primers provided with the kit. The positive and internal controls provided with the kit are aimed to prevent false negative results that may be the outcome of failed reactions or poor template quality. Using H_2O as a negative control aims to prevent false positive results by the detection of cross contamination.

Sample Material:

The **onkotest R6010-20** kit is intended for the accurate detection of EML4/ ALK (v1,v2,v3a/b) variants in bone marrow or peripheral blood samples (EDTA - ethylenediaminetetraacetic acid is the preferred as anticoagulant, heparin may inhibit the PCR reaction) of patients. The kit uses cDNA (converted from total RNA by reverse transcription) as template for the PCR reactions. Using patient RNA directly as template will not yield results.

Number of Tests:

The **onkotest R6010-20** kit contains the reaction mix, primers enough for EML4/ALK (v1,v2,v3a/b) and internal control Beta-Actin reactions of 20 patient samples. The kit also provides the reaction mix, primers enough for 4 positive control and 4 negative control reactions.

Handling & Storage

The components of kit should be stored at -200 C. While setting up PCR reactions, kit components should be placed on ice. Multiple freeze-thaw cycles may result in the degradation of protein reagents.

IMPORTANT: In order to prevent cross contamination, the positive control tube inside the kit should be stored separately from test samples and added to the reaction mix in a separate area during assay preparation.

Materials Required But Not Provided

Equipment

Real-time thermal cycler
Laminar flow hood/biological safety cabinet
Vortex
Spin-down microcentrifuge
Micropipettes

Consumables

Sterile filtered pipette tips
Serological pipettes
0,2 ml PCR tubes/Capillary tubes/strip tubes/plates (depending on the real-time instrument)

Important Notes & Precautions:

- Separate areas dedicated for RNA isolation, cDNA/PCR reaction preparation and agarose gel electrophoresis is

strongly recommended.
- Lab coats and safety equipment (goggles ect.) should be specifically designated to each area
- Separate micropipette sets for each area is strongly recommended.
- Micropipette tips should be DNAse - RNAse free and preferably filtered.
- In order to prevent cross contamination between patient samples, total RNA isolation of samples should be performed separately.
- Avoid biohazard exposure by observing universal precautions when handling all biological materials.

Before You Begin

Before starting be sure the tube contents are fully dissolved. Mix tube contents briefly by vortexing, followed by a spin-down centrifugation to bring down tube contents. Keep all test samples and kit contents on ice when in use.

Procedure

Negative control (NC) - (dH2O): This control replaces template cDNA with water in the PCR reactions. It aims for the detection of any cross contamination. The negative control should not give any positive signal. In cases where a positive reaction signal is detected, EML4/ALK (v1,v2,v3a/b) reactions must be repeated. The kit provides EML4/ALK (v1,v2,v3a/b) primers enough for 4 reactions.

Positive control (PC): Tube number 3 contains the positive control template which is the product of the EML4/ALK v1,v2 and v3b. It is expected to give 146, 183, 166 bp size products on agarose gel electrophoresis respectively. (figure 1.) EML4/ALK detection **MUST BE** positive when this control is used. Otherwise all reactions for all samples must be repeated. To avoid false positive results resulting from cross contamination we strongly suggest the addition of positive control template in to its reaction mix in a separate area. The kit provides positive

control template enough for 4 reactions. This positive control template should not be diluted when used.

.

Patient Sample Internal Control (IC)-(Beta-Actin): The Beta-Actin gene product is used as internal control. cDNA from patient samples are used as template for this reaction. The PCR product size is 202 bp. Patient sample PCRs that are negative for Beta-Actin, designate poor template quality (RNA and/or cDNA unsuitable for analyses). In case of a negative internal control (negative result for Beta-Actin expression), a new cDNA conversion from total RNA should be performed and both EML4/ALK and Beta-Actin PCR reactions should be repeated. New RNA extraction from patient bone marrow/peripheral blood should be performed in cases where a second negative result for the internal control is obtained.

Preparation of the Real-time PCR Mix

Real-time PCR Reaction Panel for One Patient :

	Negative Control **	Positive Control	Patient Beta-Actin	Patient EML4-ALK
dH_2O (tube 5)	15 µl	13,5 µl	10 µl	10µl
Real time master mix (5X) (tube 1)	4 µl	4 µl	4 µl	4 µl
EML4-ALK primer mix (tube 2)	1 µl	1 µl	-	1 µl
Positive Control (tube 3)	-	1,5 µl	-	-
Beta Actin Primer mix (tube 4)	-	-	1 µl	-
Template*	-	-	5 µl	5 µl

Total reaction volume is 20 µL

* Template is the cDNA of patient

**dH_2O replaces the template

Thermal Profile for Real-Time PCR Instrument

Denaturation	**95˚C**	**5 minutes**	**1 cycle**
Reaction	95˚C	10 seconds	25 cycles
	58˚C	10 seconds	
	72˚C	20 seconds	
Melting	95˚C 65˚C 95˚C	0 seconds 15 seconds 0 seconds	1 cycle
Cooling	40°C	30 seconds	1 cycle

After preparing the reaction mixture, the PCR tubes are placed in the real time PCR instrument and the beforementioned profile is run.

Evaluation:

After preparing the reaction mixture, the PCR tubes are placed in the real time PCR instrument and the before-mentioned profile is run. The EML4/ALK (v1,v2,v3a/b) analyses are performed after the PCR reactions. The amplification products are run on a 2% agarose gel. 5 µl of each sample and of DNA marker should be mixed with 1 µl of 6x loading dye (not provided) and be loaded onto a 2% agarose gel (not provided) for at least 45 minutes at 110 Volts or until the DNA bands are seperated from each other.

PCR products sizes are variant1 146 bp, variant2 183 bp, variant3a 132 bp and 3b 166 bp. The sizes of the PCR products are shown in figure 1.

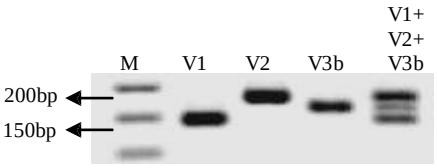

Figure 1: EML4-ALK electrophoresis of PCR product (V1+V2+V3b positive control, M-50 bp DNA marker)

Product Spesifications

Kit Capacitiy	20 samples
Control Gene	Beta-Actin
Reported Values	EML4/ALK (V1,V2,V3a/b)
Components	PCR Master mix EML4-ALK primer mix Beta-Aktin Primer mix Positive Control
Tested Platforms	Roche® LC480, LightCycler® 1.5, 2.0; ABI® StepOnePlus™; Corbett® Rotor-Gene® 6000
Product Order No	R6010-20

CONTACT:

ONKO TEST GENETİK ARAŞTIRMA PROJELENDİRME SANAYİ VE TİCARET LTD.ŞTİ.

Dokuz Eylül Üniversitesi, Depark Sağlık Zeytin binası No:56/20 ofis:202 Balçova / İZMİR

Tel: 90-232-4642088

90-216-5732591

info@onkotest.com

The following symbols may appear on the packaging and labeling:

CE	**CE sign**
	**Manufacturer**
	**Consult instructions for use**
IVD	**In vitro diagnostic**
	**Manufacturing date**
REF	**Catalog number**
LOT	**Lot number**
-22°C -18°C	**Temperature limitation**
	**Expiration date**

KKR6010-20/02.2015/00 CE