FLT3-ITD REAL TIME PCR DETECTION KIT ONKOTEST

R3030-20

Keep the kit at -15°C to -25°C

Rev. 1.5

www.onkotest.com

Product Information

FLT3 (fms-related tyrosine kinase 3) gene frequently mutated in acute myeloid leukemia (AML) is associated with disease pathogenesis. Interna tandem duplications in juxtamembran region of cytoplasmic domain of FLT3 gene is observed in 17-34% of leukemic blast cells of AML patients. Onkotest R3030-20 kit is specifically designed to detect those duplications. Onkotest R3030-20 kit cannot be used detect point mutations (D835/I836) observed in 7% of AML patients.

Kit Contents

Tube	Labeling	Volume& Content
1	Real Time Master Mix (2X)	500 µl (2X) -enough for 50 reactions. Ready to use real time PCR reaction mix.
2	FLT3-ITD Primer Mix	40 µl-enough for 20 patient samples, 5 positive control and 5 negative control reactions.
3	Positive Control	25 µl-contains a positive control template enough for 5 reactions.
4	Beta Actin Primer Mix	20 µl-enough for real time reactions of 20 patient samples.
5	H2O (PCR grade)	500 µl-Nuclease free. PCR grade. Use for reaction mixture preparation and as template for negative control reactions.

The onkotest R3030-20 kit is designed to work with all real time thermal cycler instruments.

Kit Description

The **onkotest R3030-20** kit uses cDNA of patients (converted from total RNA by reverse transcription) as template for PCR reactions. Real time PCR will amplify target the FLT3-ITD 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 R3030-20** kit is intended for the accurate detection of FLT3-ITD transcripts 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 R3030-20** kit contains the reaction mix, primers enough for FLT3-ITD and internal control Beta-Actin reactions of 20 patient samples. The kit also provides the reaction mix, primers enough for 5 positive control and 5 negative control reactions.

Handling & Storage

The components of kit should be stored at -20C°. 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 (NTC) -(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, **FLT3-ITD** reactions must be repeated. The kit provides FLT3-ITD primers enough for 5 NTC reactions.

Positive control (PC): Tube number 3 contains the positive control template which is the product of the FLT3 normal and ITD 27 aa repeat. It is expected to give 366, 447 bp size products on agarose gel electrophoresis respectively. FLT3-ITD 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 5 reactions. This positive control template should not be diluted when used. We recommend the positive control to be evaluated after the 8th cycle. Signal observed at lower cycle numbers should be evaluated as false positive.

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 FLT3-ITD 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 FLT3-ITD
dH_2O (tube 5)	9 µl	4 µl	4 µl	4 µl
Real time master mix (5X)(tube 1)	10 µl	10 µl	10 µl	10 µl
FLT3-ITD primer mix (tube 2)	1 µl	1 µl	-	1 µl
Positive Control (tube 3)	-	5 µl	-	-
Beta-Actin primer mix (tube 4)	-	-	1 µl	-
Template*	**	-	5 µl	5 µl

Total reaction volume 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	40 cycle
	56˚C	15 seconds	
	72˚C	20 seconds	
Melting	95˚C	0 seconds	1 cycle
	65˚C	15 seconds	
	95˚C	0 seconds	
Cooling	30 °C	1 minutes	1 cycle

After preparing the reaction mixture, the PCR tubes are placed in the real time PCR instrument and the before-mentioned 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 FLT3_ITD 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.
The ITD region of the FLT3 gene may have different repeat numbers. Most often these are 9 and 27 amino acid repeats. The PCR product from healthy subjects is 366 bp (with the kit PCR primer mix). In ITD positive subjects the 366 bp band and a different size band (which came from the internal tandem dublicated of the FLT3 allel) are seen together on the gel (Figure 1). The sizes of the PCR products are shown in Table 1 below:

Table 1: FLT_ITD PCR product sizes

	PCR size
Normal	366 bp
ITD 9 aa repeat	393 bp
ITD 27 aa repeat	447 bp

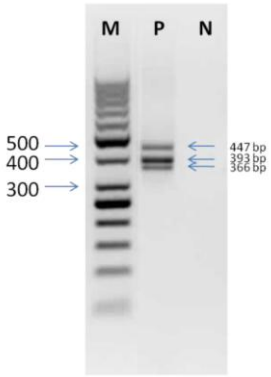

Figure 1: FLT3_ITD electrophoresis of PCR product (M=Marker, N= Negatif Control, P= repeat containing positive sample)

Product Spesifications

Kit Capacity	20 samples
Control Gene	β-Actin
Reported Values	FLT3-ITD Transcript (Normal, 9 aa and 27 aa repeat)
Components	FLT3-ITD primer mix β-Actin primer mix DNA Plasmid Control (FLT3-ITD)
Tested Platforms	Roche® LC480, LightCycler® 1.5, 2.0; ABI® StepOnePlus™; Corbett® Rotor-Gene® 6000
Product Order No.	R3030-20

CONTACT:

Ferhatpaşa Mh., 31. Sk. No:52/54 Ataşehir-İSTANBUL

Tel: 0 (216) 5732591
Fax: 0(216) 5730883