FLT3 Conventional PCR Detection Kit

ONKOTEST K3030-20

onkotest

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 K3030-20 kit is specifically designed to detect those duplications and also can be used detect point mutations (D835/I836) observed in 7% of AML patients.

Kit Contents

Tube	Labelling	Volume
1	D835I Reaction Mix (5X)	105 µl
2	D835I Restriction Enzyme Mix	105 µl
3	D835I Positive Control	18 µl
4	ITD Reaction Mix (5X)	105 µl
5	ITD Positive Control	18 µl
6	Negative Control	18 µl
7	Beta-Actin Reaction Mix (5X)	105 µl
8	Nuclease- free ddH_2O	500 µl

The onkotest K3030-20 kit is designed to work with all thermal cycler instruments.

Kit Description

The **onkotest K3030-20** kit uses cDNA of patients (converted from total RNA by reverse transcription) as template for

PCR reactions. Conventional PCR will amplify target the FLT3-ITD, D835I mutation 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 H2O as a negative control aims to prevent false positive results by the detection of cross contamination.

Sample Material:

The **onkotest K3030-20** kit is intended for the accurate detection of FLT3-ITD transcripts and point mutations (D835/I836) 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, D835I mutation 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

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, reactions must be repeated.

Positive controls (PC): Tube number 3 contains the positive control template which is the product of the D835I (D^{835}/ I^{836}) mutation. D835I mutation detection **MUST BE** positive when this control is used. After PCR amplification, it is expected to give 114 bp size product. After restriction enzyme digestion , this control displaying a completely digested product (with two smaller bands of 68 and 46 bp) corresponded to thewild-type form. Tube number 5 contains the positive control template which is the product of the FLT3-ITD respectively. The products of the FLT3- ITD are 9 aa repeat and ITD 27 aa repeat. It is expected to give 393, 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 4 reactions. This positive control template should not be diluted when used. The amplification products are run on a 2% agarose gel.

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 122 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 D835 I mutation, 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 Conventional PCR Mix

Conventional PCR Reaction Panel for One Patient:

	D835I Positive Control	Patient D835I	FLT3-ITD Positive Control	Patient ITD	Patient Beta-Aktin	Negative Control
ddH_2O (tube 8)	12 µl	12 µl	12 µl	12 µl	12 µl	12 µl
D835I Reaction mix (5X) (tube 1)	4 µl	4 µl	-		-	-
D835I Positive Control (tube 3)	4 µl	-	-		-	-
ITD Reaction mix (5X) (tube 4)	-	-	4 µl	4 µl	-	-
ITD Positive Control (tube 5)	-	-	4 µl		-	-
Beta-Aktin Reaction mix (5X) (tube 7)	-	-	-		4 µl	4 µl
Negative Control (tube 6)	-	-	-	-	-	4 µl
Template*	-	4 µl	-	4 µl	4 µl	-

*Template is the cDNA of the patient
Total reaction volume is 20 µL

Thermal Profile for the Thermal Cycler Instrument

Denaturation	95˚C	5 minute	1 cycle
Reaction	95˚C	30 second	40 cycles
	56˚C	45 second	
	72˚C	30 second	
extention	72˚C	5 minute	1 cycle

After preparing the reaction mixture, the PCR tubes are placed in the thermal cycler instrument and the before mentioned profile is run.

D^{835}/ I^{836} Restriction Enzyme Digestion

D835I PCR product obtained from after PCR amplification use as a template* for digestion to determine whether the D835I mutation. Protocol as the following:

	D835I Patient Rest. digestion
ddH_2O (tube 8)	1 µl
D835I Rest. Enz. (tube 2)	4 µl
D835I PCR Product (template*)	15 µl
Total volume	20 µl

Incubate at 37°C for 1 hour. After incubation the digestion products should be run on a 2% agarose gel for evaluation.

Evaluation:

After preparing the reaction mixture, the PCR tubes are placed in the thermal cycler instrument and the before-mentioned profile is run. The FLT3_ITD analyses are performed after the PCR reactions. The D835I mutation analyses are performed after restriction enzyme digestion. The products are run on a 2% agarose gel. 5 µl of each sample for ITD and of DNA marker should be mixed with 1 µl of 6x loading dye (not provided) and 15 µl of each sample for the D835I digest product and of DNA marker should be mixed with 3 µl

of 6x loading dye 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.

According to the results should be evaluated by agarose gel electrophoresis is they are positive or negative reactions . Onkotest K3030-20 kit is specifically designed to detect FLT3-ITD and also can be used detect point mutation (D835 / I836).

PCR reactions are performed to determine D835I point mutation in patient samples. The products (114 bp) obtained after the PCR reaction cut with the restriction enzyme reaction . If samples displaying a completely digested product (with two smaller bands of 68 and 46 bp) corresponded to the wild-type (normal) form. If uncut 114 bp bands observed it is indicated that the presence of the D835 / I836 mutations.

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).

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

Product Spesifications

Kit capasity	20 samples
control Gene	Beta-Actin
Reported values	FLT3-ITD Transcript (9 aa ve 27 aa repeat) D^{835}/ I^{836} point mutation
Components	D835I Reaction mix D835I Rest. Enzyme mix FLT3-ITD Reaction mix Beta-Actin Reaction mix Positive Controls (D835I and FLT3-ITD) Negative control
Tested Platforms	Ependorf Thermal Cycler, Techne Thermal Cycler
Product order no	K3030-20

CONTACT

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CE	CE sign
	Manufacturer
	Consult instruction for use
IVD	in vitro diagnostic
	Manufacturing date
REF	Catolog number
LOT	LOT number
-18°C -22°C	Temperature limitation
	Expiration date

KK3030-20/22.10.2015/00

CE