Genmark Sağlık Ürünleri
İTH. İHR SAN. TİC. LTD.

Instructions For Use - version 1.2.2

CE IVD

1.1, 1.2, 1.3, 1.4, 1.5, 1.6 pirkimo dalys
Rinkinys žymėtas CE-IVD.

For In Vitro Diagnostic Use

Store at -25°C to -15°C

REF

AML1-RT24 / t (8;21) (q22;q22) RUNX1-RUNX1T1 (AML1-ETO) **E2A-RT24 /** t (1;19) (q23;p13) TCF3/PBX1 **MLL-RT24 /** t (4;11) (q21;q23) MLL-AF4 **TEL-RT24 /** t (12;21) (p13;q22) TEL-AML1 **CBF-RT24 /** Inv(16) (p13q22) CBFB-MYH11 **PML-RT24 /** t (15;17) (q22;q21) PML-RARA bcr1& bcr2, bcr3	Σ	24
BCR190-RT48 / t(9;22) (q34:q11) minor BCR-ABL1 (p190) e1a2 **BCR230-RT48 /** t(9;22) (q34:q11) micro BCR-ABL1 (p230) e19	Σ	48

geneMAP™ Translocation Detection Kits for Leukemia

For Real-Time PCR

Kits for quantitation of fusion transcripts of AML1-ETO, TCF3/PBX1, MLL-AF4, TEL-AML1, CBFB-MYH11, PML-RARA bcr1&bcr2 and bcr3, minor BCR-ABL1(p190) e1a2, micro BCR-ABL1(p230) e19a2.

Validated on:

* Biorad® CFX96, Real-time PCR System (Bio-Rad)
* Life Technologies ABI Prism® - 7500, Step-One & QuantStudio Series
* Qiagen Rotor-Gene® 3000 Q5/Q6
* Roche, LightCycler® 480 II, Cobas Z480
* BioMolecular Systems, MicPCR
* BaseTyper™, Pentabase

1.1 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis AML1-ETO (RUNX1-RUNX1T1) transkriptus.
1.2 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis E2A(sinonimas: TCF3)-PBX1 transkriptus
1.3 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis MLL-AF4 transkriptus.
1.4 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis PML-RARA bcr 1, 2, 3 transkriptus.
1.5 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis TEL-AML1 transkriptus.
1.6 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis CBFB-MYH11 transkriptus.

Contents

1. Intended Use

The Translocation Detection Kits for Leukemia are intended for the quantitative detection of the following fusion transcripts in bone marrow or peripheral blood samples.

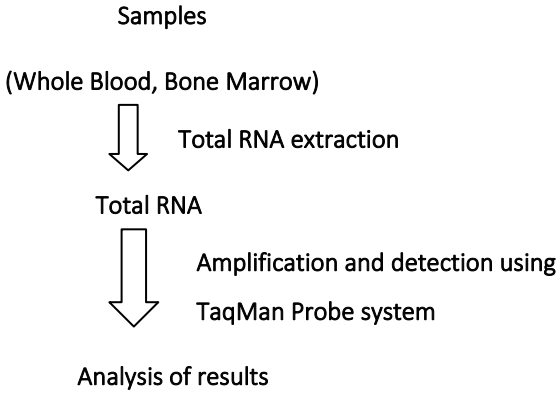
AML1-ETO, TCF3/PBX1, MLL-AF4, TEL-AML1, CBFB-MYH11, PML-RARA bcr1&bcr2 and bcr3, minor BCR-ABL1(p190) e1a2, micro BCR-ABL1(p230) e19a2.

2. Principles and Procedure Overview

2.1 Principles

The polymerase chain reaction (PCR) is the sensitive and specific TaqMan Probe technology with the use of DNA amplification technique, primer design and PCR optimization. The Kit is based on two main processes: nucleic acid extraction and PCR amplification of nucleic acid in the primer and probe mechanism of PCR machines by real time PCR. The Translocation Detection Kits are a PCR test where fusion transcripts and ABL1 targets are able to ensure amplification of nucleic acids in a single step.

Procedure Overview;



2.2 Technology

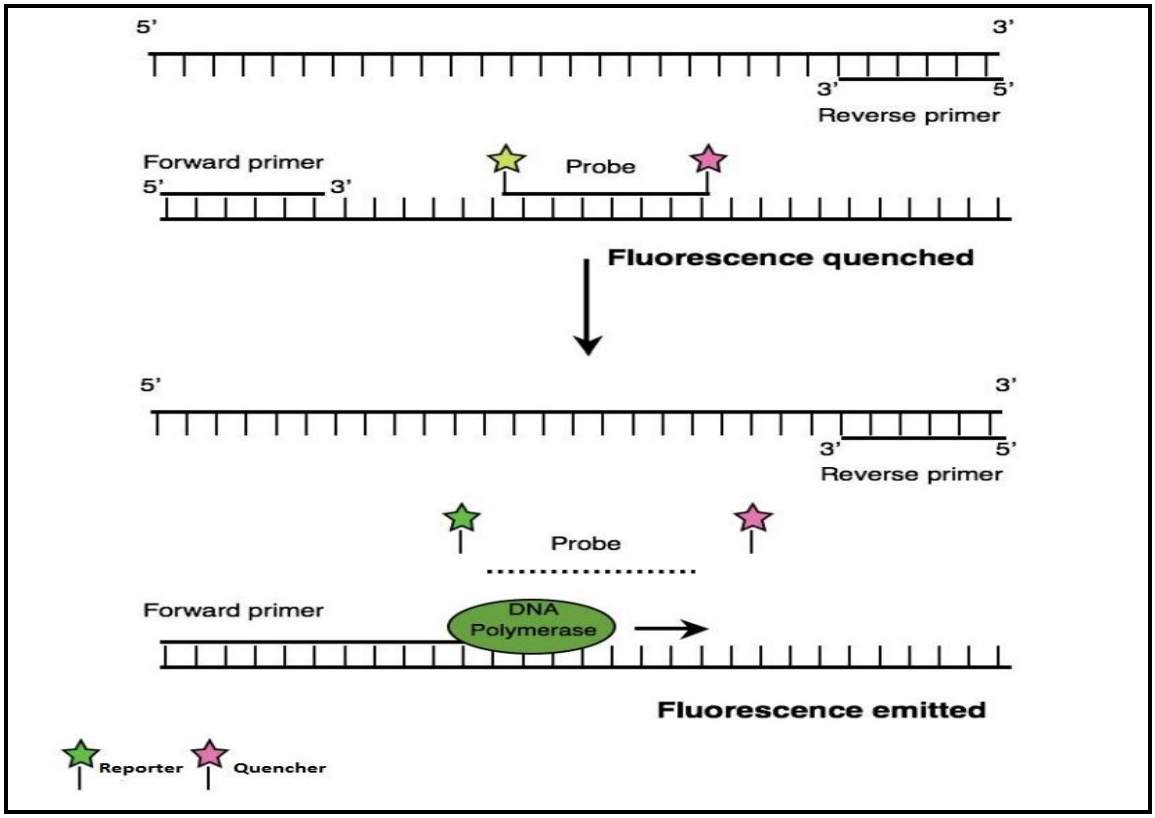
Hydrolysis (TaqMan) probes are the most common form of qPCR probes and are widely used in human, veterinary, and environmental diagnostics. These probes utilize a fluorescent dye at one end of the DNA oligonucleotide and a quencher at the other. During PCR, the probe specifically anneals to the target DNA sequence (from sample), which is flanked by the two primers. As DNA polymerase extends the new DNA strand, the probe is degraded by the 5' to 3' exonuclease activity of the polymerase, resulting in the fluorophore being separated from the quencher and emitting fluorescence. The more DNA is present in the reaction, the earlier the fluorescence reaches a detectable level resulting in earlier Ct values.

This kit utilizes primer sets and a FAM-labeled hydrolysis probe to detect and quantify the presence of the fusion transcript. The primer mix also contains primers and a Cal Fluor® Orange 560 (VIC equivalent)-labeled probe that detect the amplification of ABL as an internal control. This enables the kit to use the Delta Ct method (Livak Method) to determine the target-reference gene ratios.

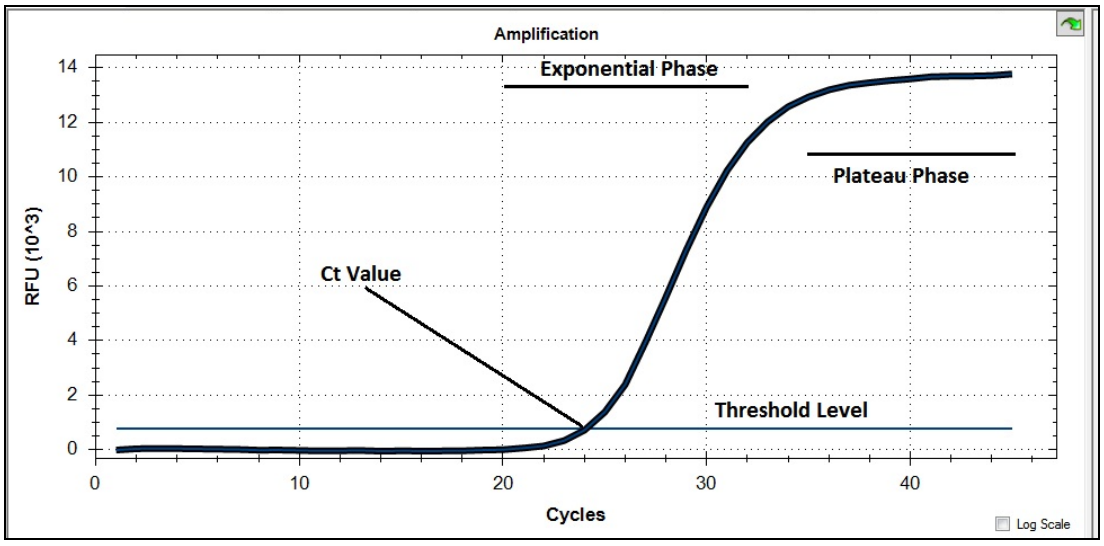
This kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. A combination of validated primers, hydrolysis probes, and hot-start DNA polymerase ensures that the Translocation Detection Kits for Leukemia leads to highly-specific and ultra-sensitive results in a single step.

The resulting Ct values or copy numbers of Fusion transcript and ABL1 transcripts are used with the provided formulas to calculate the Fusion Gene Transcript/ABL ratios (%).

1.1, 1.2, 1.3, 1.4, 1.5, 1.6 pirkimo dalys
Atvirkštinė transkripcija ir kiekybinis vertinimas vykdomas tame pačiame mėgintuvėlyje, neperkeliant mėginio.



Hydrolysis (TaqMan) Probe Technology.



Typical Amplification Plot of Real-Time PCR in Linear Scale Graphic

This kit utilizes primer sets and a FAM-labeled hydrolysis probe to detect and quantify the presence of the fusion transcript. The primer mix also contains primers and a Cal Fluor® Orange 560 (VIC equivalent)-labeled probe that detect the amplification of ABL1 as an endogenous control. This enables the kit to use the Delta Ct method (Livak Method) or Absolute Quantification Method to determine the target-reference gene ratios.

3. Background Information

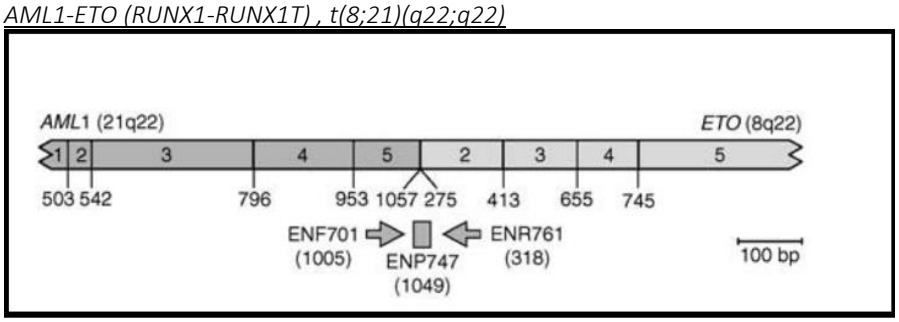
Leukemia is one of the most common cancer types worldwide, presenting 250,000 cases annually. Leukemia is described as a malignant disease caused by abnormal white bloodcells produced in bone marrow. An exacerbated and uncontrolled production of abnormal blood cells occurs, leading to a decreased production of healthy blood cells, promoting the rise of bleeding, several infections and severe anemia. In addition, leukemic cells can also spread to other organs such as spleen, brain, lymph nodes and other tissues. (1, 2)

Chromosomal translocations result in creation of gene fusion transcripts that aberrantly modulate various cellular processes. The table below lists translocations that are commonly associated with childhood and adult leukemias.

Translocation	Fusion Gene	Diagnosis	Frequency
t(8;21)(q22;q22)	AML1-ETO (RUNX1-RUNX1T)	Acute myeloid leukemia (AML)	8%
t(1;19)(q23;p13)	TCF3/PBX1	Childhood precursor-B-ALL Adult precursor-B-ALL	3-5% 3%
t(4;11)(q21;q23)	MLL-AF4	Childhood and adult precursor-B-ALL	5%
t(12;21)(p13;q22)	TEL-AML1	Childhood precursor-B-ALL	25%
Inv(16) (p13q22)	CBFB-MYH11	Acute myeloid leukemia (AML)	8-9%
t(15;17)(q22;q21)	PML-RARA bcr1, bcr2, bcr3	Acute Promyelocytic Leukemia (APL)	95%
t(9;22)(q34:q11)	Minor BCR-ABL1 (p190)	Acute Lymphoblastic Leukaemia (ALL) or Chronic Myeloid Leukemia (CML)	5% (Pediatrics) 5-50%(Adults)
t(9;22)(q34:q11)	Micro BCR-ABL1 (p230)	Acute Lymphoblastic Leukaemia (ALL) or Chronic Myeloid Leukemia (CML)	1-3%

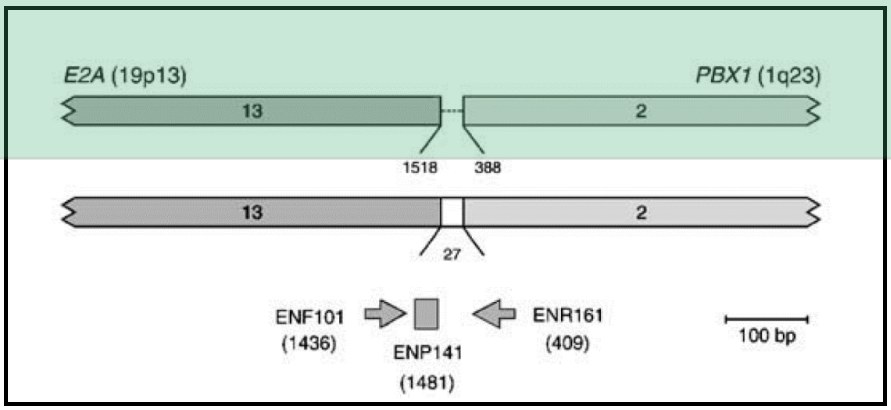
Breakpoint types of fusion genes which can be detected by the kits;

1) *AML1-ETO (RUNX1-RUNX1T) , t(8;21)(q22;q22)*



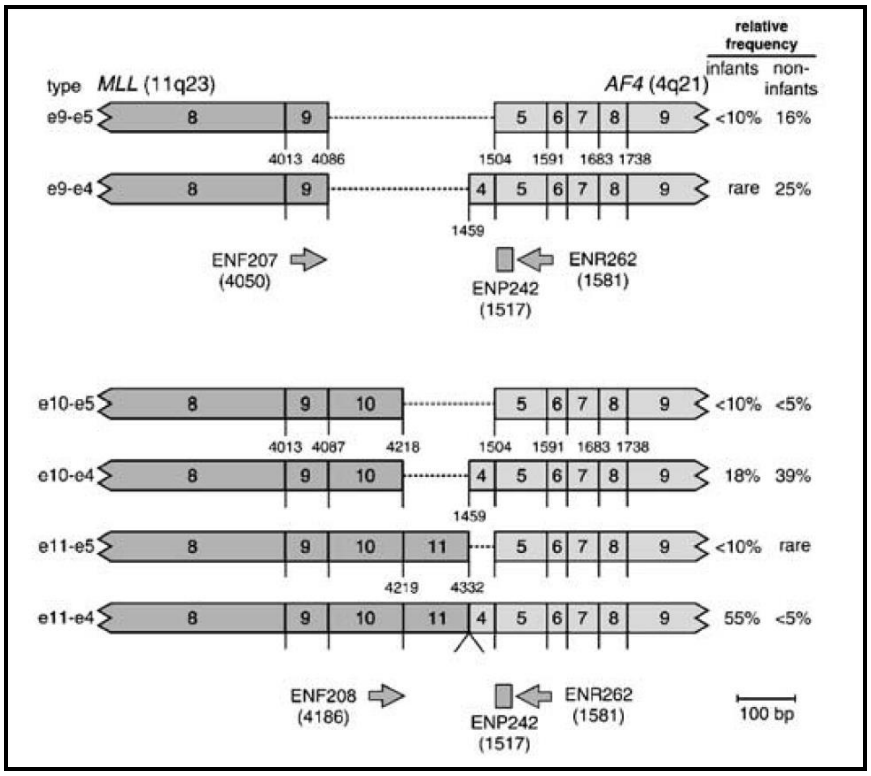
1.2 pirkimo dalis
Tikro laiko PGR rinkinys, kiekybiškai nustatantis E2A(sinonimas: TCF3)-PBX1 transkriptus

2) TCF3/PBX1 *t(1;19)(q23;p13)*

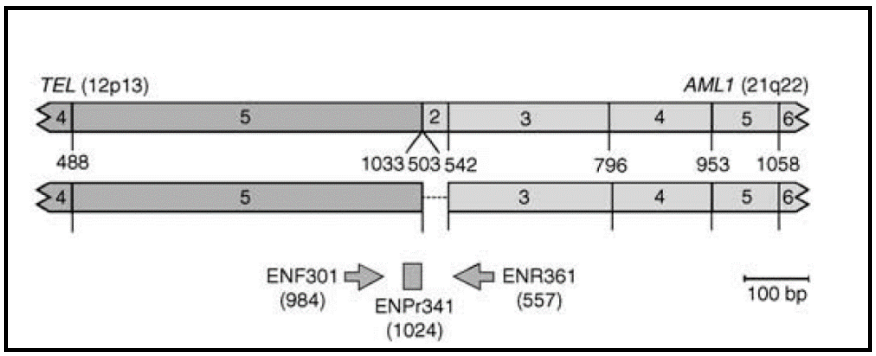


3) *MLL-AF4 t(4;11)(q21;q23)*

- *TYPE e9-e5, e9-e4, e10-e5, e10-e4, e11-e5, e11-e4*

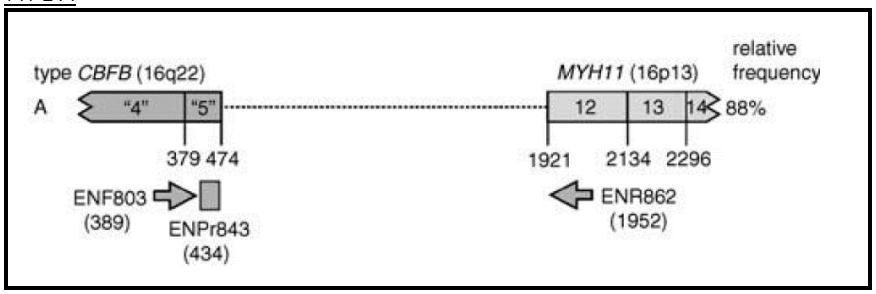


4) *TEL-AML1 t(12;21)(p13;q22)*



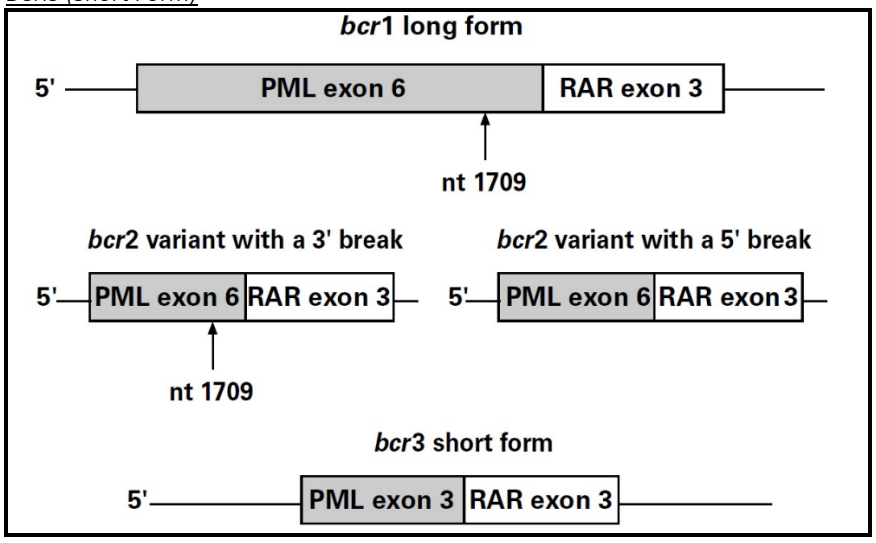
5) *CBFB-MYH11 Inv(16) (p13q22)*

- *TYPE A*

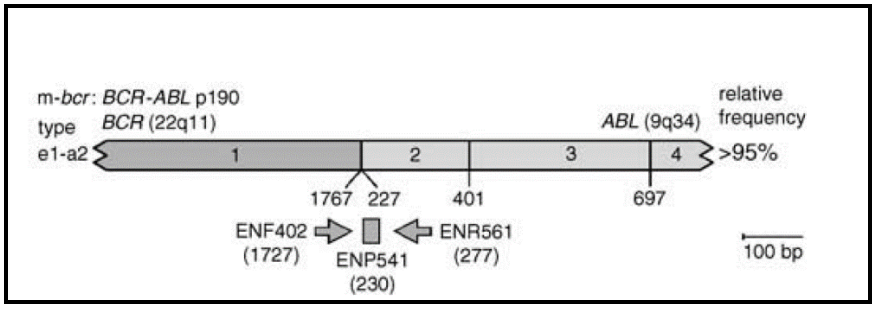


6) *PML-RARA bcr1&bcr2, bcr3-t(15;17)(q22;q21)*

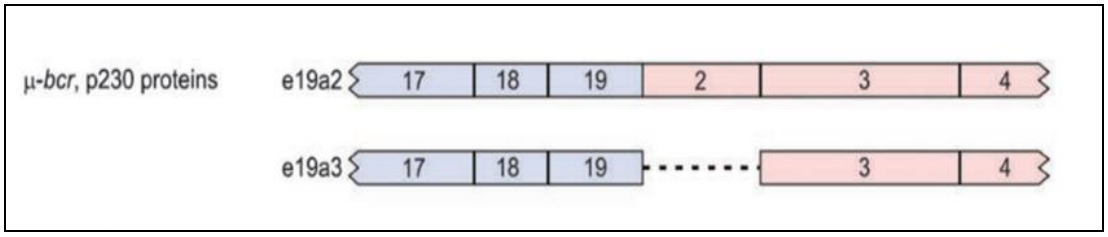
- *BCR1 (Long Form)*
- *BCR2 (Variant 3' break & Variant 5' break)*
- *BCR3 (Short Form)*



7) *Minor BCR-ABL1 (p190)- t(9;22) (q34:q11)*



8) *Micro BCR-ABL1 (p230)-t(9;22) (q34:q11)*



1.1, 1.2, 1.3, 1.5, 1.6 pirkimo dalys
Į rinkinį įeina visi reikalingi reagentai taikininio transkripto bei namų ruošos geno (angl. housekeeping gene) nustatymui naudojant prieš tai išgrynintą tiriamojo RNR. Įskaitant standartus su žinomu kopijų skaičiumi, atvirkštinės transkripcijos ir polimerazės grandininės reakcijos reagentus. Pažymėta žaliai.

4. Reagents

1.1, 1.2, 1.3, 1.5, 1.6 pirkimo dalys
Rinkinyje yra 33 reakcijos.

This product contains the following materials: Reagents contained in a kit are sufficient for 33 reactions.

Description	No. of Reactions	No. of Tubes	Vol.per. Tube (µl)	Color of Caps
5X One-Step qRT-PCR Buffer	33	1	132	Blue
OneStep qRT_PCR Enzyme Mix	33	1	53	Yellow
RNase Free Water	33	1	500	Violet
5x Translocation Primer-Probe Mix§	33	1	132	Amber
Transcript Positive Control ₸	10	1	50	Red
Calibrator	10	1	50	Orange

₸ The Positive Controls contain a mixture of plasmid sequences target and reference gene.

§ The primer probe mix tube contains primers/probes for both Leukemia fusion transcript and ABL1 control gene.

For minorP190 BCR-ABL1, micro P230 BCR-ABL1 : Reagents contained in a kit are sufficient for 66 reactions.

Description	No. of Reactions	No. of Tubes	Vol.per. Tube (µl)	Color of Caps
5X One-Step qRT-PCR Buffer	66	1	264	Blue
OneStep qRT_PCR Enzyme Mix	66	1	106	Yellow
RNase Free Water	66	1	500	Violet
5x Translocation Primer-Probe Mix§	66	1	264	Amber
Transcript Positive Control ₸	10	1	50	Red
Calibrator	10	1	50	Orange

₸ The Positive Controls contain a mixture of plasmid sequences target and reference gene.

§ The primer probe mix tube contains primers/probes for both Leukemia fusion transcript and ABL1 control gene.

1.4 pirkimo dalis
Rinkinyje yra 33 reakcijos. Pažymėta violetine spalva.

For PML-RARA Reagents contained in a kit are sufficient for 33 reactions.

Description	No. of Reactions	No. of Tubes	Vol.per. Tube (µl)	Color of Caps
5X One-Step qRT-PCR Buffer	66	1	264	Blue
OneStep qRT_PCR Enzyme Mix	66	1	106	Yellow
RNase Free Water	66	1	500	Violet
5x BCR1&2 Primer-Probe Mix§	33	1	132	Amber
5x BCR3 Primer-Probe Mix§	33	1	132	Amber
Transcript Positive Control ₸	10	1	100	Red
Calibrator	10	1	100	Orange

₸ The Positive Controls contain a mixture of plasmid sequences target and reference gene.

§ The primer probe mix tube contains primers/probes for both Leukemia fusion transcript and ABL1 control gene.

5. Storage and Handling

All components of the kit must be stored at between -15°C /-25°C. All components are stable under the recommended storage conditions until the expiration date indicated on the label on the box. The performance of the kit components is not affected until 5 freeze and thaw. If reagents are to be used only intermittently, they should be stored in aliquots.

6. Materials Required But Not Provided

- Bleach (2-10%)
- Disposable powder free gloves (latex ornitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5mL microcentrifuge tubes
- Desktop centrifuge
- Vortex mixer

1.4 pirkimo dalis
Į rinkinį įeina visi reikalingi reagentai taikininio transkripto bei namų ruošos geno (angl. housekeeping gene) nustatymui naudojant prieš tai išgrynintą tiriamojo RNR. Įskaitant standartus (kalibratorius) su žinomu kopijų skaičiumi, atvirkštinės transkripcijos ir polimerazės grandininės reakcijos reagentus. Pažymėta geltona spalva.

- Clean bench

7. Protocol

7.1 Specimen Collection, Storage, and Transport

All samples should be considered as potentially infectious material. Only sample materials collected, stored and transported in accordance with the following rules and instructions are permitted.

To ensure high sample quality, samples should be transported as quickly as possible. The samples should be transported at the specified temperatures.

7.1.1 Specimen Collection

Obtaining peripheral whole blood must be collected in EDTA.

7.1.2 Specimen Storage and Transport

It is recommended to minimize sample handling, to ship specimens (i.e. whole blood) on cold packs pulled from storage at 2 to 8°C, to store the specimen at 2 to 8°C once received in the laboratory, and to process the specimens within 48 hours of collection.

Specimen	Storage[1]		Transport[2]	Note
	Temp.	Duration	Temp.	
Whole Blood in EDTA	2-8°C	Max 2 days	2-8°C	Never freeze/thaw the sample
Bone marrow				

Note1: Performance may be affected by prolonged storage of specimens.

7.2 Nucleic Acid Extraction

7.2.1 Pre-analytical Steps

Total RNA extracted via common sample preparation methodologies from whole blood collected in EDTA is compatible with RT-qPCR methods. In general, it is recommended that a minimum of 10 and a target of 20 million nucleated cells be collected, enriched, and extracted into RNA at a target concentration of 50-500 ng/µL. Since RNA is notoriously subject to degradation by ubiquitous RNases present in human specimens, RNA quality and quantity can greatly affect the results. The purified total RNA must be evaluated for concentration (OD260 indicating a concentration of ≥50 ng/µL) and purity (as estimated by OD260/OD280 ratio>1.6) by standard spectrophotometric methods. To facilitate rapid and efficient test setup, purified total RNA should be tested immediately or in accordance with laboratory preparation methodology as validated with this test by the laboratory.

Category	Specification
Whole blood	Variable (2-5mL)
Nucleated cell	Target of 20 million (2×10^7)
RNA concentration	50-500 ng/µL
RNA purity	OD_{260}/ OD_{280} ratio >1.6

7.2.2 Sample Enrichment (White Blood Cell Isolation)

Sample requirement for optimal results is fresh (no freeze-thawed) 2-5mL Peripheral Blood or 500uL-1mL bone marrow.

1. Dilute the 10X Red Blood Cell (RBC) Lysis Buffer (necessary but not provided with the kit) to 1X working concentration with deionized water if necessary.
2. Add to sample (blood or bone marrow) into Conical Tube (Falcon 15ml or 50mL) containing RBC buffer 3-4 times more than sample volume.

(Example; 3ml Blood+12ml 1xRBC buffer or 0,5mL bone marrow +2ml 1X RBC buffer)

3. Gently whirl the conical tube immediately after adding the samples into the RBC Lysis Buffer, incubate at room temperature for 10-15 minutes.
4. Centrifuge the conical tubes at 600 g for 10min.
5. Discard the supernatant without disturbing the pellet.
6. Pour 1mL 1x RBC Lysis Buffer into the pellet and mix by pipetting several times until dissolving all pellet and transfer all mixture into the new 1,5mL microcentrifuge tube.
7. Incubate for 3 min. at room temperature.
8. Centrifuge the Eppendorf tube in 3000 rpm for 2 minutes.
9. Discard the supernatant carefully. Pellet consists of White Blood Cells.

Note: Pellet (White Blood Cells) must be used immediately for RNA extraction step. Avoid freezeing-thawing of WBC pellet.

Note: Please use the recommended volumes of specimen and elution as indicated below. For all others, refer to the manufacturer's protocol.

Extraction Kit	Manufacturer	Cat.No	Recommended Elution Vol.
QIAamp® RNA Blood Mini Kit	QIAGEN	52304	Elution:50 µL
Hybrid R Blood (Total RNA Extraction Kit)	GeneAll	315-150	Elution:50 µL

Follow the total RNA extraction procedure according to the manufacturer's protocol. This assay can detect the fusion gene in a broad range of total RNA (250-2500ng). It is important to measure the RNA concentration using spectrophotometric analysis (i.e. Nanodrop or UV spectrophotometer) in order to prevent over/under- loading of the reactions. A recommended starting concentration is 50-500 ng/µL.

7.3 Preparation for Real-time PCR

Note: The correct tubes and caps must be used (see MATERIALS REQUIRED BUT NOT PROVIDED).

Note: Aerosol resistant filter tips and tight gloves must be used when preparing one-step RTPCR reactions. Pay extreme attention to ensure no cross-contamination.

Note: Thaw all reagents completely on ice.

Note: Set up all reactions on ice to minimize the risk of RNA degradation.

Note: Centrifuge the reagent tubes for short to remove drops from the inside of the cap.

1. Prepare the one-step RT-PCR Mastermix

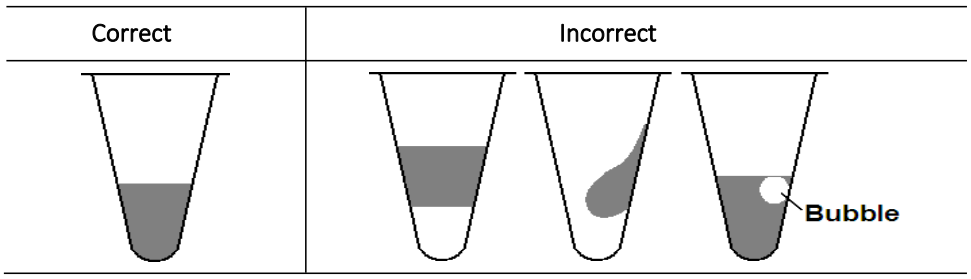
5X Translocation PPM	4 µl
5X OneStep PCR Buffer	4 µl
RNase Free Water	5,4 µl
OneStep Enzyme Mix	1,6 µl
Total volume of PCR Mastermix	15 µl

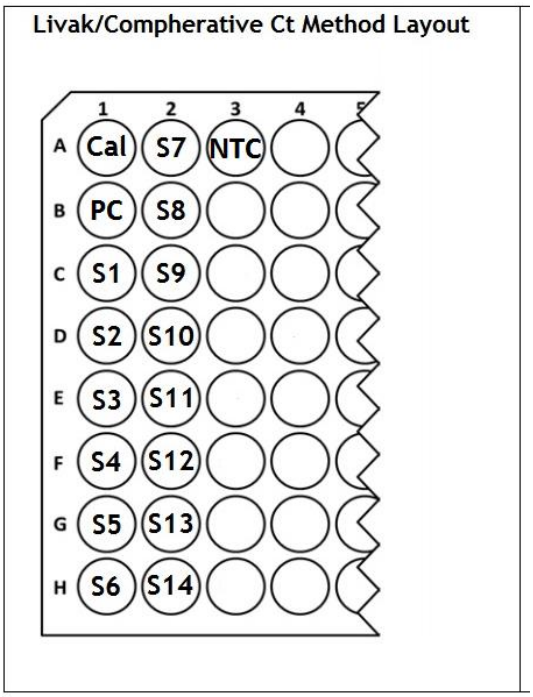
Note: Calculate the necessary amount of each reagent needed based on the number of reactions (samples + controls).

2. Mix by inverting the tube 5 times or quick whirl, and centrifuge for short.
3. Aliquot 15 µL of the one-step RT-PCR Mastermix into PCR tubes.
4. Add 5 µL of each sample's nucleic acids into the tube containing the one-step RT-PCR Mastermix.

PCR Mastermix	15 µl
Sample's nucleic acid, PC, Cal, Std's	5 µl
Total volume of reaction	20 µl

Note: Use a new sterile pipette tip for each sample.
Note: For Negative Control (NC), use 5 µL of RNase-free Water instead of sample's nucleic acid.
Note: For Positive Control (PC), use 5 µL of PC.
Note: Please be careful not to cross-contaminate the one-step RT-PCR Mastermix and samples with the Positive Control.
Note: The PCR tubes must be mixed and centrifuged before running PCR reaction. It needs to be checked that liquid containing all PCR components is at the bottom of each PCR tube.





Example Plate Layout for both Livak (Comparative Ct).

8. Real-Time PCR Instrument Setup and Results Analysis

8.1 Real-time PCR System

8.1.1 Pre-settings for Data Analysis

A. Pre-settings for Reporter (Florophore) Selection and Thermal Cycling Conditions

Analyte	Reporter
Translocation	FAM
ABL1	CalFlour Orange 560/VIC/HEX

Temperature	Time	Cycles	Data Collection
50˚C	30 min	1	
95˚C	15 min	1	
95˚C	15 sec	45	
62˚C	1 min		FAM, VIC

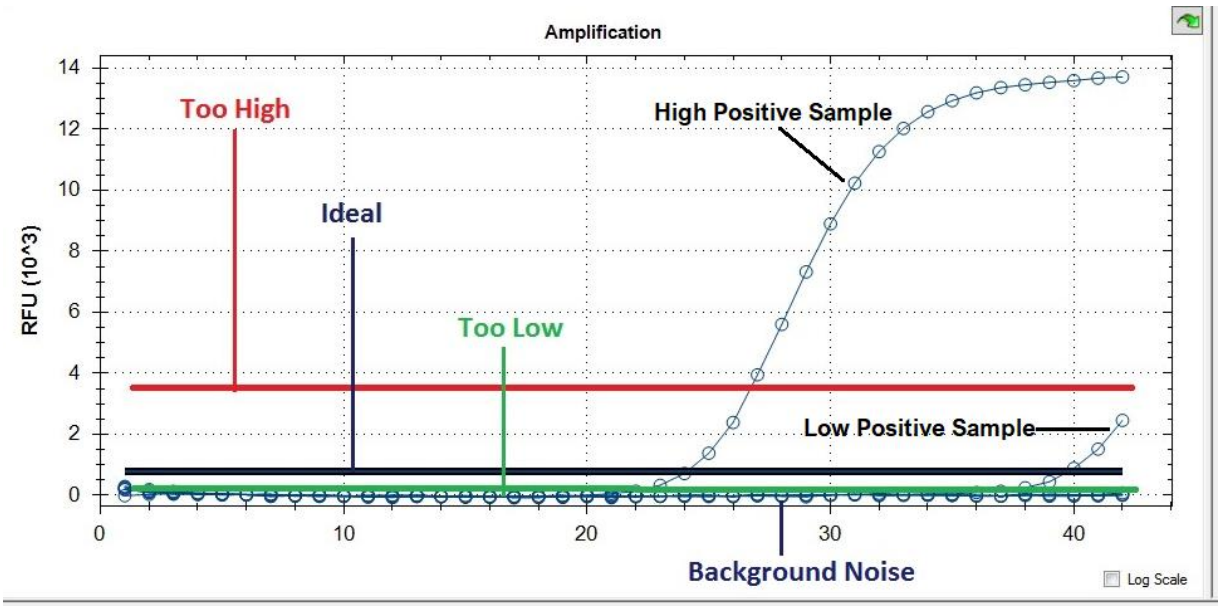
Note: *Please use only 72-well carousel, 36-well carousel does not recommended and perform Auto-Gain optimisation befor first acquisition (Auto-Gain optimisation tube should be PC) For Rotorgene Q5/Q6.*

9. Results

9.1 General Rules of the Threshold Settings Manually

Normally the software-based methods will select a proper threshold, but in cases where the curves do not conform to the assumptions made by the algorithm, an incorrect threshold may be calculated. Good indicators of improperly set threshold values are false positives (Ct values obtained from negative control wells), known positive samples giving very late Cts or no Cts at all. Because of those reasons adjusting the threshold manually is highly recommended.

Ideally, the threshold should be set in the region just above the backround noise. The threshold should not be so high that it crosses any of the plots where they start to plateau and are no longer linear.



Example of ideal threshold level.

If threshold is too high, it gives false negative (Missing the low positive sample).

If threshold is too low, it gives false positive (Intercept the background noise).

9.2 Interpretation of Results

Analyte	Fluorophore	Sample	
		Ct Value	Result
Translocation	FAM	<40	Positive (+)
ABL1	CalFlour Orange 560/VIC/HEX	≤24	Positive (+)
		>24 or NA	Negative (-)

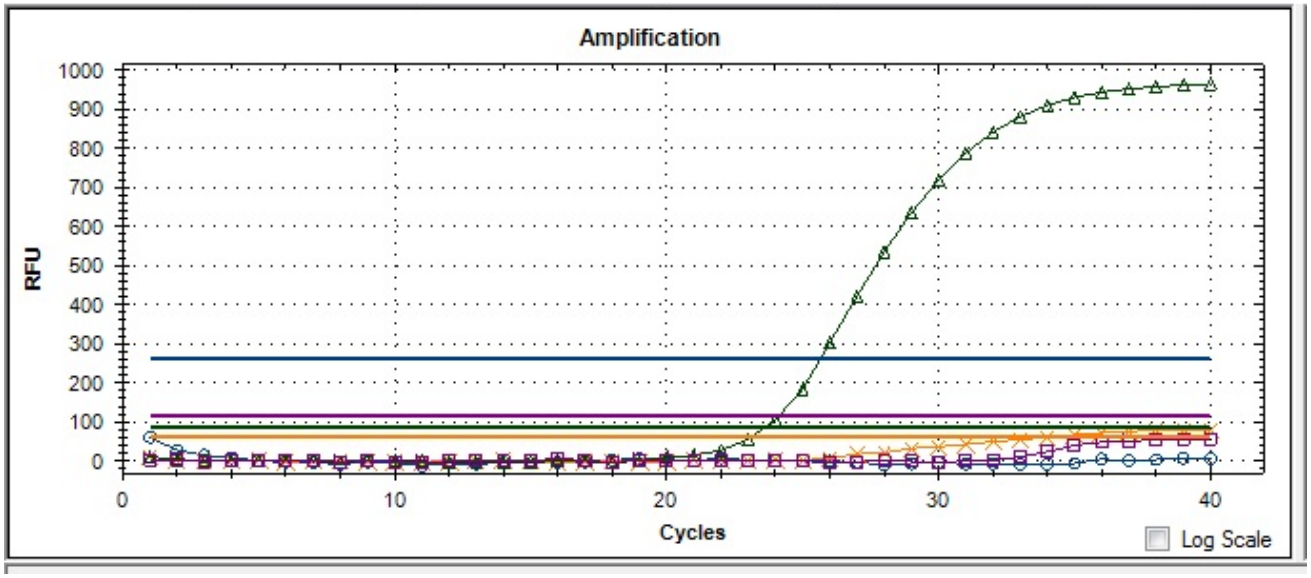
	FAM (Translocation)	VIC (ABL1)	Interpretation	
Case 1	+	+	FAM: Detected VIC:Detected	Translocation Positive. **Calculate the ratio.**
Case 2	-	+	FAM: Not Detected VIC:Detected	Negative
Case 3	-	-	FAM: Not Detected VIC:Not Detected	Invalid

Fusion Gene Positive Results Criteria			
Translocation	FAM Ct	VIC Ct	Transcript Status
t(8;21)(q22;q22) RUNX1-RUNX1T1 (AML1-ETO)	20≤Ct≤40	19≤Ct≤24	Positive
t(1;19)(q23;p13) TCF3/PBX1	20≤Ct≤40	19≤Ct≤24	
t(4;11)(q21;q23) MLL-AF4	20≤Ct≤40	19≤Ct≤24	
t(12;21)(p13;q22) TEL-AML1	20≤Ct≤40	22≤Ct≤24	
Inv(16) (p13q22) CBFB-MYH11	20≤Ct≤38	22≤Ct≤24	
t(15;17) (q22;q21) PML-RARA bcr1, bcr2, bcr3	Bcr1&2=20≤Ct≤40 Bcr3=20≤Ct≤40	19≤Ct≤24	
t(9;22) (q34:q11) minor BCR-ABL1 (p190) e1a2	20≤Ct≤40	18≤Ct≤24	
t(9;22) (q34:q11) micro BCR-ABL1 (p230) e19a2	20≤Ct≤40	18≤Ct≤24	

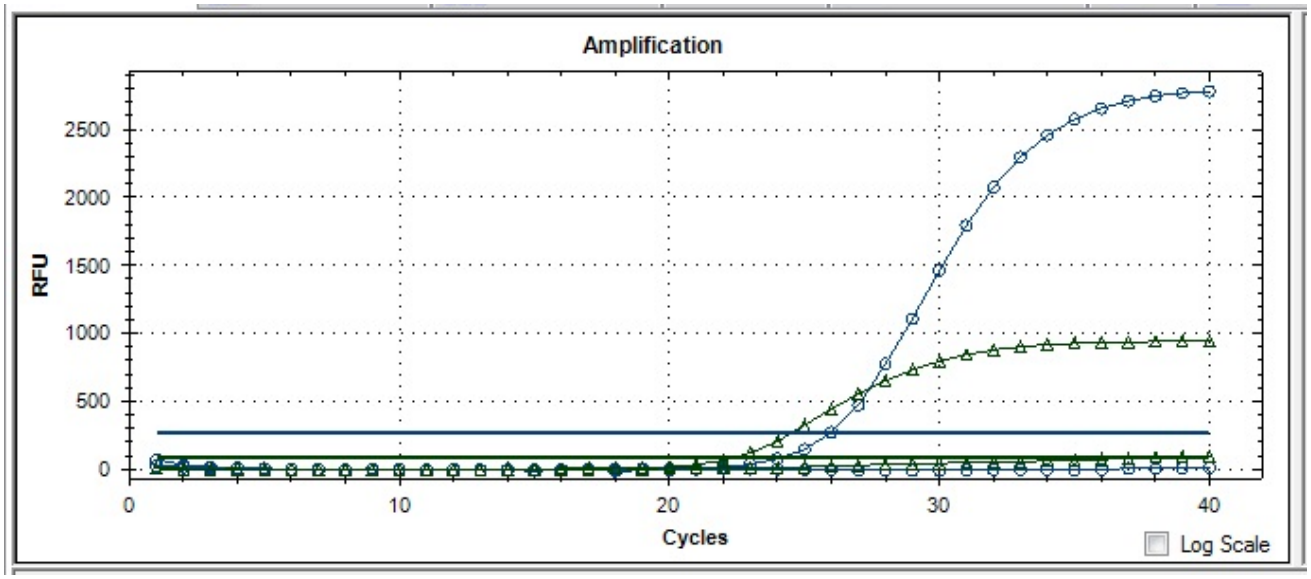
Fusion Gene Negative Results Criteria			
Translocation	FAM Ct	VIC Ct	Transcript Status
t(8;21)(q22;q22) RUNX1-RUNX1T1 (AML1-ETO)	Ct >40 or N/A	19≤Ct≤25	Not Detected
t(1;19)(q23;p13) TCF3/PBX1	Ct >40 or N/A	19≤Ct≤25	
t(4;11)(q21;q23) MLL-AF4	Ct >40 or N/A	19≤Ct≤25	
t(12;21)(p13;q22) TEL-AML1	Ct >40 or N/A	23≤Ct≤29	
Inv(16) (p13q22) CBFB-MYH11	Ct >38 or N/A	22≤Ct≤26	
t(15;17) (q22;q21) PML-RARA bcr1&bcr2, bcr3	Bcr1&2=Ct>40 or N/A Bcr3=Ct>40 or N/A	19≤Ct≤25	
t(9;22) (q34:q11) minor BCR-ABL1 (p190) e1a2	Ct >40 or N/A	18≤Ct≤24	
t(9;22) (q34:q11) micro BCR-ABL1 (p230) e19a2	Ct >40 or N/A	18≤Ct≤24	

Fusion Gene Invalid Results Criteria			
Translocation	FAM Ct	VIC Ct	Interpretation
t(8;21)(q22;q22) RUNX1-RUNX1T1 (AML1-ETO)	Ct >40 or N/A	Ct>25	Insufficient RNA/Repeat the test with more RNA
t(1;19)(q23;p13) TCF3/PBX1	Ct >40 or N/A	Ct>25	
t(4;11)(q21;q23) MLL-AF4	Ct >40 or N/A	Ct>25	
t(12;21)(p13;q22) TEL-AML1	Ct >40 or N/A	Ct>29	
Inv(16) (p13q22) CBFB-MYH11	Ct >38 or N/A	Ct>26	
t(15;17) (q22;q21) PML-RARA bcr1& bcr2, bcr3	Bcr1&2=Ct>40 or N/A Bcr3=Ct>40 or N/A	Ct>25	
t(9;22) (q34:q11) minor BCR-ABL1 (p190) e1a2	Ct ≥40 or N/A	Ct>25	
t(9;22) (q34:q11) micro BCR-ABL1 (p230) e19a2	Ct ≥40 or N/A	Ct>25	

Some Example Results:



Translocation not detected: There is only VIC (ABL1) Curve (Triangle).



Translocation Positive:There are FAM Curve (Circle) and VIC (ABL1) Curve (Triangle).

9.1 Settings for Livak/Comparative Ct Method

Use the real-time instrument software and set the threshold manually and perform the analysis.
Note all Ct values of PC, Calibrator and Samples.

9.1.1 Calculation of Normalized Copy Number (NCN)

Perform the International Scale Normalized Copy Number (NCN-IS) for each sample using Microsoft Excel or other spreadsheet applications using the following formula:

$$NCN\ (\%) = \frac{1{,}9^{\text{Delta Ct (Sample or Calibrator)}}}{2^{\text{Delta Ct (PC)}}} \times 100$$

Where: Delta Ct=ABL1 Ct — BCR/ABL1 Ct

$$\text{Convertion Factor} = \frac{\text{NCN (\%) calibrator (Lot Specific)}}{\text{NCN (\%) calibrator (Calculated)}}$$

$$\text{NCN normalized (\%)} = \text{NCN Patient x Conversion Factor}$$

Note: Please find the Calibrator NCN (Lot Specific) value on the Calibrator Tube label in the kit box.

10. Performance of the Kit

The analytical performance of this assay was assessed on a Biorad CFX96 instrument synthetic long RNA sequences encoding each breakpoint region of the respective fusion genes, as well as RNA samples previously assessed for presence of the fusion genes. Bio-RAD CFX96, Roche LightCycler 480 and ABI 7500 devices were used in the studies. It has the sensitivity to detect 5 copies of the BCR-ABL transcript in 50,000 ABL transcripts. (10^{-4}= LOG 4)

Linearity:

AML1-RT24 / t (8;21) (q22;q22) RUNX1-RUNX1T1 (AML1-ETO): The Kit linearity is in a range from 0.01 to 89 NCN (%)
E2A-RT24 / t (1;19) (q23;p13) TCF3/PBX1**:** The Kit linearity is in a range from 0.01 to 79 NCN (%).
MLL-RT24 / t (4;11) (q21;q23) MLL-AF4: The Kit linearity is in a range from 0.01 to 82 NCN (%).
TEL-RT24 / t (12;21) (p13;q22) TEL-AML1: The Kit linearity is in a range from 0.01 to 82 NCN (%).
CBF-RT24 / Inv(16) (p13q22) CBFB-MYH11: The Kit linearity is in a range from 0.01 to 78 NCN (%).
PML-RT24 / t (15;17) (q22;q21) PML-RARA bcr1& bcr2: The Kit linearity is in a range from 0.01 to 80 NCN (%).
bcr3: The Kit linearity is in a range from 0.01 to 78 NCN (%).
BCR190-RT48 / t(9;22) (q34:q11) minor BCR-ABL1 (p190) e1a2: The Kit linearity is in a range from 0.01 to 90 NCN (%).
BCR230-RT48 / t(9;22) (q34:q11) micro BCR-ABL1 (p230) e19a2: The Kit linearity is in a range from 0.01 to 85 NCN (%).

11. Quality Control Criteria

Minimum Ct level for ABL1 transcripts for valid result	≤24	The test must be repeat or perform the RNA extraction from fresh new sample
Maximum Ct level for ABL1 transcripts for valid result	≥18 (≥16 for QiagenQ5/Q6 Series instrument)	RNA must be dilute as 1/5-1/10 and repeat the test.
Range of Conversion Factor for proper NCN calculation	Should be between 0.4 to 2.4	The test must be repeat

12. Clinical Valiation Studies

* AML1-RT24 / geneM.A.P™ RUNX1-RUNX1T1 t(8;21) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ RUNX1-RUNX1T1 t(8;21) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/Ul

Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ RUNX1-RUNX1T1 t(8;21) Detection Kit		Negative	Positive	Total
	Negative	112	0	112
	Positive	0	19	19
	Total	112	19	131

Statistic	Value	95% CI
Sensitivity	100.00%	82.35% to 100.00%
Specificity	100.00%	96.76% to 100.00%
Accuracy	100.00%	97.22% to 100.00%

* E2A-RT24 / geneM.A.PTM TCF3 / PBX1 t(1;19) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ TCF3 / PBX1 t(1;19) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

Test Name	**Comparator Test Q (CE-IVD Marked qRT-PCR Kit)**			
geneM.A.P™ TCF3 / PBX1 t(1;19) Detection Kit		Negative	Positive	**Total**
	Negative	112	0	**112**
	Positive	0	8	**8**
	Total	**112**	**8**	**120**

Statistic	Value	95% CI
Sensitivity	100.00%	82.35% to 100.00%
Specificity	100.00%	96.76% to 100.00%
Accuracy	100.00%	97.22% to 100.00%

* MLL-RT24 / geneM.A.PTM MLL-AF4 t(4;11) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ MLL-AF4 t(4;11) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ MLL-AF4 t(4;11) Detection Kit		Negative	Positive	Total
	Negative	112	0	112
	Positive	0	15	15
	Total	112	15	127

Statistic	Value	95% CI
Sensitivity	100.00%	78.20% to 100.00%
Specificity	100.00%	96.76% to 100.00%
Accuracy	100.00%	97.14% to 100.00%

* TEL-RT24 / geneM.A.PTM TEL-AML1 t(12;21) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ TEL-AML1 t(12;21) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ TEL-AML1 t(12;21) Detection Kit		Negative	Positive	**Total**
	Negative	112	0	**112**
	Positive	0	9	**9**
	Total	**112**	**9**	**121**

Statistic	Value	95% CI
Sensitivity	100.00%	66.37% to 100.00%
Specificity	100.00%	96.76% to 100.00%
Accuracy	100.00%	97.00% to 100.00%

* CBF-RT24 / geneM.A.PTM CBFB-MYH11 Inv(16) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ CBFB-MYH11 Inv(16) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ CBFB-MYH11 Inv(16) Detection Kit		Negative	Positive	**Total**
	Negative	112	0	**112**
	Positive	1	15	**16**
	Total	**112**	**15**	**128**

Statistic	Value	95% CI
Sensitivity	93.75%	69.77% to 99.84%
Specificity	100.00%	96.76% to 100.00%
Accuracy	99.22%	95.72% to 99.98%

* PML-RT24 / geneM.A.PTM PML-RARA t(15;17) bcr1&bcr2, bcr3 Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneM.A.P™ PML-RARA t(15;17) bcr1&bcr2, bcr3 Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.

Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

- **Break bcr1**

Test Name	**Comparator Test Q (CE-IVD Marked qRT-PCR Kit)**			
geneM.A.P™ PML-RARA t(15;17) bcr1&bcr2, bcr3 Detection Kit		Negative	Positive	**Total**
	Negative	54	1	**55**
	Positive	0	14	**1**
	Total	**55**	**15**	**70**

Statistic	**Value**	**95% CI**
Sensitivity	100.00%	76.84% to 100.00%
Specificity	98.18%	90.28% to 99.95%
Accuracy	98.55%	92.19% to 99.96%

- **Break bcr2**

Test Name	**Comparator Test Q (CE-IVD Marked qRT-PCR Kit)**			
geneM.A.P™ PML-RARA t(15;17) bcr1&bcr2, bcr3 Detection Kit		Negative	Positive	**Total**
	Negative	54	0	**54**
	Positive	0	3	**3**
	Total	**54**	**3**	**57**

Statistic	**Value**	**95% CI**
Sensitivity	100.00%	29,24% to 100.00%
Specificity	100.00%	93.40% to 100.00%
Accuracy	100.00%	93.73% to 100.00%

- **Break bcr3**

Test Name	**Comparator Test Q (CE-IVD Marked qRT-PCR Kit)**			
geneM.A.P™ PML-RARA t(15;17) bcr1&bcr2, bcr3 Detection Kit		Negative	Positive	**Total**
	Negative	54	0	**54**
	Positive	0	9	**9**
	Total	**54**	**9**	**63**

Statistic	**Value**	**95% CI**
Sensitivity	100.00%	66.37% to 100.00%
Specificity	100.00%	93.40% to 100.00%
Accuracy	98.55%	92.19% to 99.96%

* BCR190-RT48 / geneM.A.PTM BCR-ABL1 P190 (mbcr) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneMAP™ BCR-ABL1 p190 (mbcr) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.
Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ BCR-ABL1 P190(mbcr) Detection Kit		Negative	Positive	Total
	Negative	149	0	149
	Positive	1	16	17
	Total	150	16	166

Statistic	Value	95% CI
Sensitivity	100.00%	79.41% to 100.00%
Specificity	99.33%	96.34% to 99.98%
Accuracy	99.40%	96.69% to 99.98%

* BCR230-RT48 / geneM.A.PTM BCR-ABL1 P230 (µbcr) Detection Kit

The clinical performance of the assay was established in one site clinical evaluation. Clinical specimens were tested with geneMAP™ BCR-ABL1 p230 (mbcr) Detection Kit and a commercial qRT-PCR based assay which has CE-IVD marked choosen as a comparator. Results are summarized below.
Note1: The insturment is CFX96/ Biorad
Note2: RNA's concentration are between 20-350 ng/uL

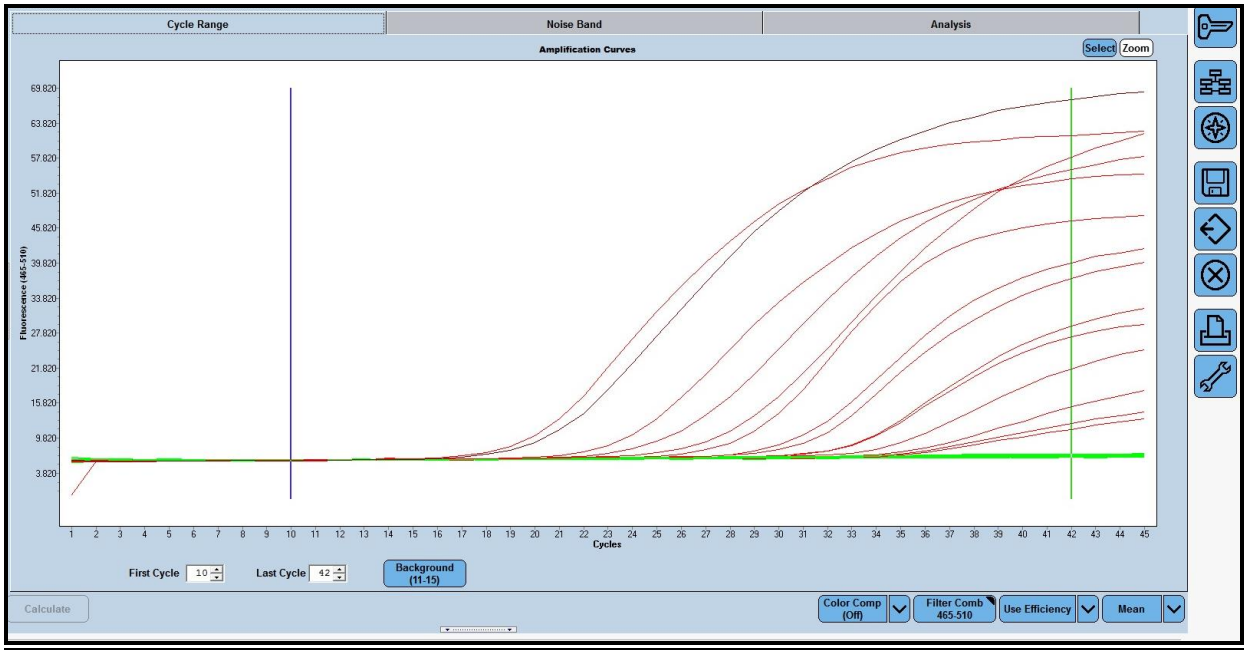
Test Name	Comparator Test Q (CE-IVD Marked qRT-PCR Kit)			
geneM.A.P™ BCR-ABL1 P230(µbcr) Detection Kit		Negative	Positive	Total
	Negative	150	0	150
	Positive	0	12	12
	Total	150	12	162

Statistic	Value	95% CI
Sensitivity	100.00%	73.54% to 100.00%
Specificity	100.00%	97.57% to 100.00%
Accuracy	100.00%	97.75% to 100.00%

13. Technical Notes

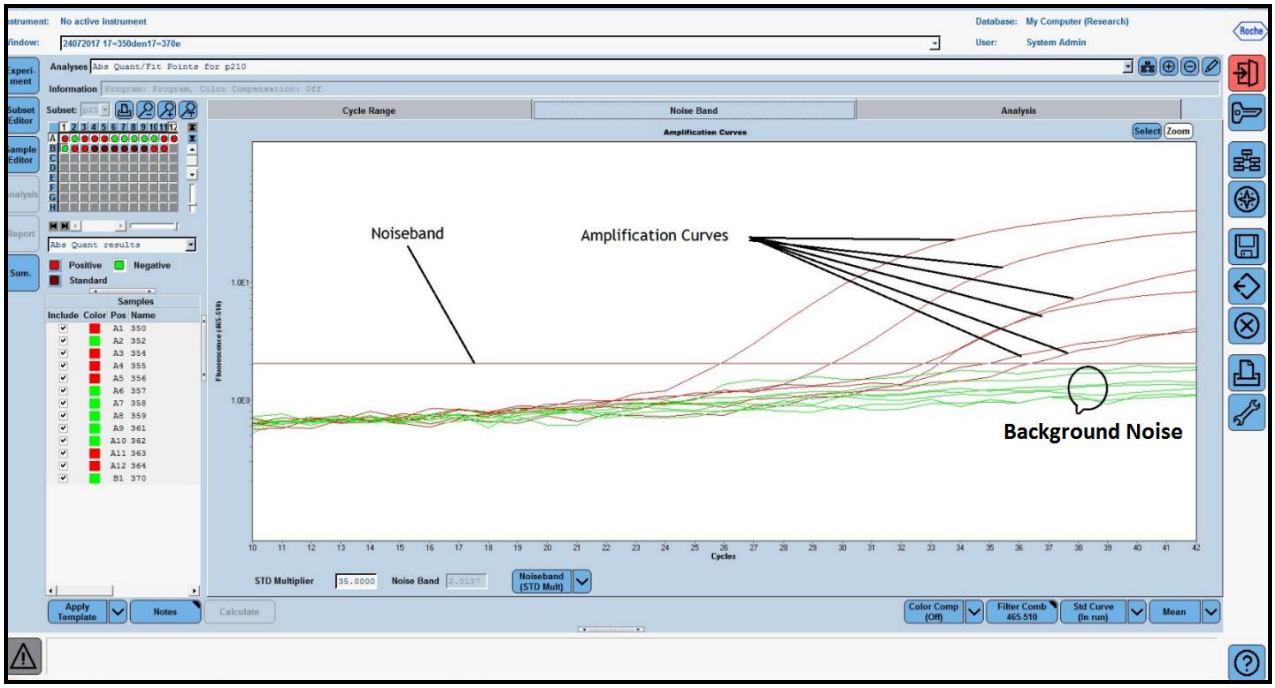
Please refer to following additional information if you are using LightCycler480 (ROCHE) Realtime PCR insturment
Livak /Comparative Ct Method
Follow the steps below for each of the detectors. Analyze FAM first, then VIC.

1. In the **sample editor** assign sample names.
2. Select **Analysis** button, **Abs Quant/Fit Points**.
3. In the **Cycle Range** tab.



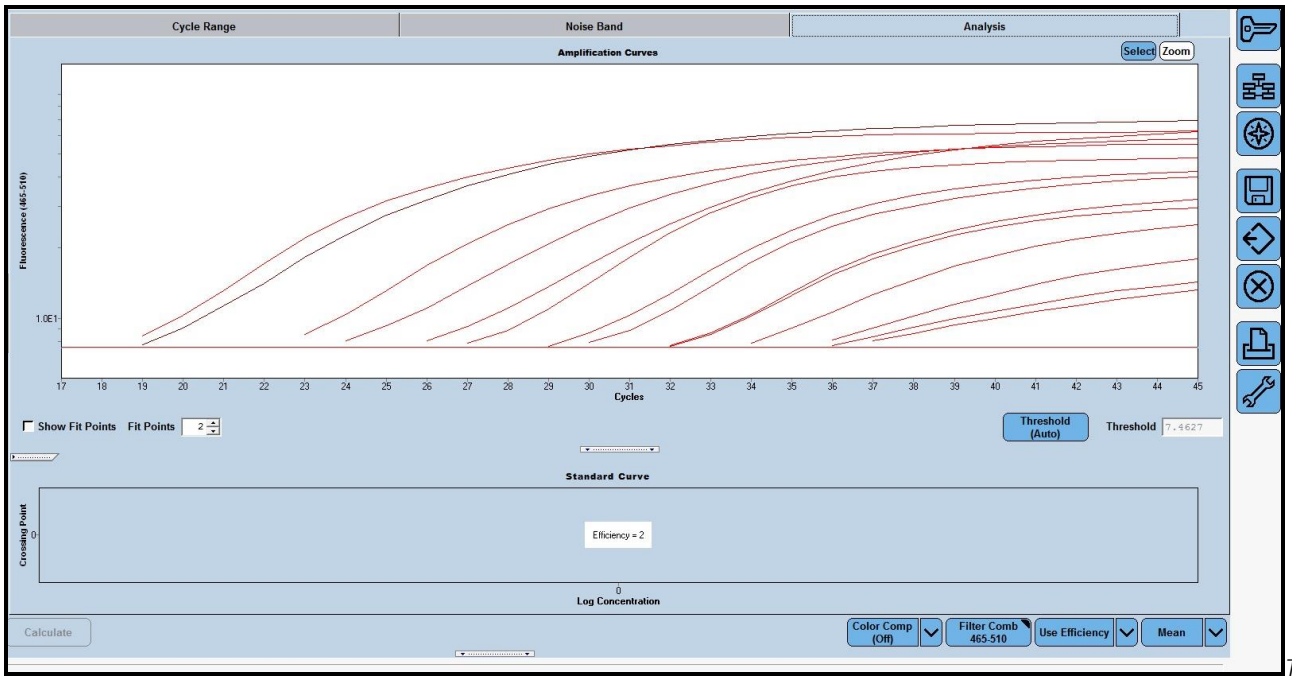
*Select detectors by clicking on **Filter Comb** button. Select FAM (465-510) Choose First cycle: 10 for FAM Last Cycle: 42*

4. Select **Noise Band** Tab.



The Noise Band line is manually dragged over directly to just above the Background Noise.
(STD Multiplier value can be between 20-40 depending on the instrument performance)

5. Select **Noise Band** Tab.



Tt

Threshold setting must be Auto Mode

14. References

1-https://www.mayoclinic.org/diseases-conditions/leukemia/symptoms-causes/syc-20374373

2- Gabert J et al., Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program, Leukemia (2003) 17, 2318–2357

15. Revision History

Date of Last Edit: December 2023		
Change	**Affected Section**	**Page**
Added reaction numbers for the kit	Reagents	8
Added ∑ (total numbers of tests) symbol	Cover Page	1
Some information is changed	7.1Nucleic Acid Ectracition	10
Correction Misspelling	9.1.1Calculation of Normalized Copy Number (NCN)	17

16. Troubleshooting

Translocation Detection Kits for Leukemia		
OBSERVATION	PROBABLE CAUSES	SOLUTION
No signal	The fluorophores for data analysis does not comply with the protocol	Select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 9) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Presence of inhibitor	Please dilute (1/10~1/100) the template nucleic acid with RNase-free Water and repeat the test with the diluted nucleic acid.
Putative false positive or target signals observed in Negative Control	Contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Use filter tips only and throughout the procedure and change tips between tubes. Repeat the entire procedure from nucleic acid extraction with the new set of reagents.
False negative or no signal observed in Positive Control	The fluorophores for data analysis does not comply with the protocol	Please select the correct fluorophores for data analysis.
	Incorrect setting of real-time thermal cycler	Please check the thermal cycling conditions and repeat the test under the correct settings.
	Incorrect PCR mixture	Confirm that all components are added to the PCR mixture. Sensitivity is compromised with pre- composed premix. All reagents must be homogenized and spun down before use.
	Did not add sample's nucleic acid	Please repeat the test carefully.
	Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers of tubes containing nucleic acid and make sure to add nucleic acid into the correct PCR tubes and repeat the test carefully if necessary.
	Incorrect storage or past expiry date of the test kit	Please check the storage condition (See page 9) and the expiry date (refer to label) of the test kit and use a new kit if necessary.
	Error in nucleic acid extraction	Please check the nucleic acid extraction procedure as well as nucleic acid concentration, and re-extract the nucleic acid. If the original specimen is not available, a new specimen must be collected.
NCN Value is too High (More than 100%)	Competition between Translocation and ABL1 primer probe set. The Result is out of Linear Range of the Kit.	Some patients have too much translocation transcript thus ABL1 amplification is suppressed by translocation amplification. Report the patient NCN Translocation Detection Kits for Leukemia -IS (%): >80

17. Symbols Used

Catalog Number

Lot/Batch Number


Expiration Date

Storage Conditions


Manufactured by

Intended Use

18. Contact Information

Genmark Sağlık Ürünleri
İthalat İhracat ve Ticaret Limited Şirketi
Halil Rıfat Paşa Mah. Güler Sok. GNM Plaza No:51-1 34384 Okmeydanı / Şişli- İstanbul

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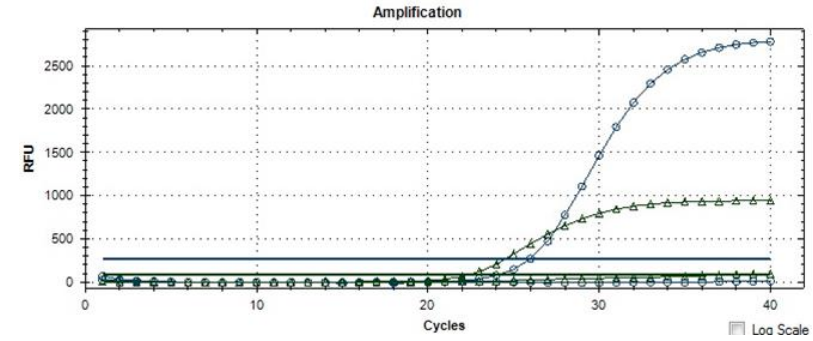
HEMATOLOGY-ONCOLOGY

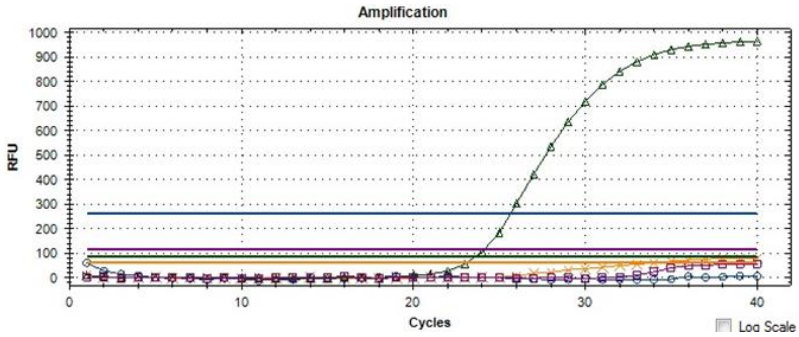
RUNX1-RUNX1T1(AML1-ETO) t(8;21), TCF3/PBX1 t(1;19), MLL-AF4 t(4;11), TEL-AML1 t(12;21), CBFB-MYH11 inv16, PML-RARA bcr1&bcr2, bcr3 t(15;17), BCR-ABL (p190), BCR-ABL1 (p230)

Leukemia Panel Translocation Detection Kit

1.1, 1.2, 1.3, 1.4, 1.5, 1.6 pirkimo dalys
Į rinkinį įeina visi reikalingi reagentai taikininio transkripto bei namų ruošos geno (angl. housekeeping gene) nustatymui naudojant prieš tai išgrynintą tiriamojo RNR.

Positive Result Analysis





Negative Result Analysis

- ✓ With One Step enzyme, sensitive and precise results are obtained.
- ✓ Quantitative determination of transcripts can be done from total RNA
- ✓ ABL is used as reference gene (housekeeping).
- ✓ Primer probe mix, target gene and reference gene (ABL) are all in one tube.
- ✓ Calibrator and conversion constant allows results to be converted to International Scale (NCN-IS)
- ✓ When enough amount of RNA is collected, the LOG (10^{-4}) decrease of fusion transcript can be detected.

GeneMAP™ Leukemia Panel Translocation Detection Kit is designed specifically for detection of translocations with routine RT-qPCR. It is easy to use, cost efficient and compatible with many Real-time PCR instruments.

Contents	Volume
5X One-Step qRT-PCR Buffer	132 µl
OneStep qRT_PCR Enzyme Mix	53 µl
RNase Free Water	500 µl
5x Translocation Primer-Probe Mix	132 µl
Transcript Positive Control	50 µl
Calibrator	50 µl

Technical Specifications

Intended Use
- For the quantitative analysis of
- AML1-ETO,
- TCF3/PBX1,
- MLL-AF4,
- TEL-AML1,
- CBFB-MYH11,
- PML-RARA bcr1&bcr2 ve bcr3,
- minor BCRABL1(p190) e1a2,
- micro BCR-ABL1(p230) e19a2 fusion transcripts.

Suitable Sample Type
-Whole Blood in tube with EDTA
-Bone Marrow

Test Procedure
-RNA Isolation
-Multiplex RT-qPCR

Analytical Sensitivity
- For each 50,000 ABL1 transcript, 5 fusion transcripts can be detected (LOG4).

Validated PCR Instruments
- Bio-Rad CFX96
- Life Technologies ABI-7500
- Roche, Light Cycler 480 II
- Qiagen Rotor-Gene® 3000 Q5/Q6
- BioMolecular Systems, MicPCR
- QuantStudio™ 5 Real-Time PCR System

Catalog Number
- AML1-RT24 / - E2A-RT24
- MLL-RT24 / -TEL-RT24
- CBF-RT24 / -PML-RT24
- BCR190-RT48
- BCR230-RT48

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Genmark Sağlık Ürünleri İTH. İHR SAN. TİC. LTD.

In **GENMARK SAGLIK URUNLERI,** we aim to create the top quality, time and cost efficient, trust-worthy and user-friendly products. We specialize in in-vitro detection kit production and development which is used for the **diagnosis** and **treatment monitoring** of many diseases connected to genetics, oncology, microbiology and hematological oncology.