Leukemia Fusion Genes (Q51) Screening Kit

This package insert must be read carefully prior to use and should be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

REF **803418**

20 Tests per Kit
For Research Use Only

Table of Contents

1. INTENDED USE

LEUKEMIA Fusion Genes (Q51) Screening Kit is a qualitative test for screening of 52 fusion genes (**Table 1**) resulted from chromosome translocations involved in chronic and acute leukemia. All the 52 fusion genes include more than 182 clinically relevant chromosomal breakpoints [1-9]. The kit detects RNA transcripts of fusion genes extracted from human bone marrow or whole blood samples using a RT-qPCR procedure. The results of the kit allow professionals to be more aware of the patient`s prognosis and provides professionals important insights into the treatment planning.

Table 1. 52 Chromosome Translocations and fusion genes in the Kit

No.	Translocation	Fusion Gene	No.	Translocation	Fusion Gene
1	t (9;11) (p22; q23)	MLL-AF9	**27**	t (3; 21) (q26; q22)	AML1-EAP
2	t (8; 21) (q22; q22)	AML1-ETO	**28**	t (6; 11) (q27; q23)	MLL-AF6
3	t (4; 11) (q21; q23)	MLL-AF4	**29**	-	EVI1
4	t (12; 21) (p13; q22)	TEL-AML1	**30**	t (9; 22) (q34; q11)	BCR-ABL1 p190
5	t (1;19) (q23; p13)	E2A-PBX1	**31**	t (9; 22) (q34; q11)	BCR-ABL1 p210
6	t (11;19) (q23; p13.3)	MLL-ENL	**32**	t (15; 17) (q24; q21)	PML-RARα S
7	del (1) (p32)	SIL-TAL1	**33**	t (15; 17) (q24; q21)	PML-RARα V
8	t (10; 11) (p12; q23)	MLL-AF10	**34**	t (15; 17) (q24; q21)	PML-RARα L
9	inv (16) (p13; q22)/ t (16;16) (p13;q22)	CBFβ-MYH11	**35**	t (10; 11) (p13; q21)	CALM-AF10
10	t (3; 21) (q26; q22)	AML1-MDS1/EV11	**36**	-	HOX11
11	del (4) (q12)	FIP1L1-PDGFRA	**37**	t (9; 12) (p24; p13)	TEL-JAK2
12	del (9) (q34)	SET-CAN	**38**	t (x; 11) (q13; q23)	MLL-AFX1
13	t (17; 19) (q22; p13)	E2A-HLF	**39**	t (4; 12) (q12; p13)	ETV6-PDFGRA
14	t (6; 9) (p23; q34)	DEK-CAN	**40**	-	HOX11L2
15	t (X; 11) (q24; q23)	MLL-SEPT6	**41**	(11q23)	dup MLL
16	t (16; 21) (p11; q22)	TLS-ERG	**42**	t (1; 11) (q23; p15)	NUP98-PMX1
17	t (5; 12) (q33; p13)	TEL-PDGFRB	**43**	t (2; 11) (q31; p15)	NUP98-HOXD13
18	t (11; 19) (q23; p13.1)	MLL-ELL	**44**	t (7; 11) (p15; p15)	NUP98-HOXA9
19	t (11; 17) (q23; q21)	MLL-AF17	**45**	t (7; 11) (p15; p15)	NUP98-HOXA13
20	t (5; 17) (q35; q21)	NPM-RARα	**46**	t (11; 12) (p15; q13)	NUP98-HOXC11-
21	t (3; 5) (q25; q34)	NPM-MLF1	**47**	t (7; 11) (p15; p15)	NUP98-HOXA11
22	t (11; 17) (q23; q21)	PLZF-RARα	**48**	der (17)/t (17; 17) (q21; q21)	STAT5-RARα
23	t (1; 11) (q21; q23)	MLL-AF1q	**49**	t (11; 17) (q13; q21)	NUMA-RARα
24	t (1; 11) (p32; q23)	MLL-AF1P	**50**	t (4; 17) (q12; q21)	FIPIL1-RARα
25	t (9; 12) (q34; p13)	TEL-ABL1	**51**	der (17)	PRKAR1A-RARα
26	t (16; 21) (q24; q22)	AML1-MTG16	**52**	t (2; 5) (p23; q35)	NPM-ALK

2. PRINCIPLE OF THE PROCEDURES

LEUKEMIA Fusion Genes (Q51) Screening Kit is a multiplex RT-qPCR based assay for detection of leukemia associated fusion gene transcripts in total RNA from bone marrow or whole blood samples. Included in the kit are RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix prepared in advance. The resulting cDNA is added to 12 qPCR reaction tubes, which contain specific PCR primers and probes for detection of fusion genes and an internal control gene of GUSB. The qPCR is performed in a real-time thermal cycler with optical filters for the detection of FAM, ROX, HEX and Cy5 fluorescence signals. Amplification plots and the resulting Cq (quantification cycle) values are used for the identification of the fusion gene transcript.

THE kit detects fusion gene transcripts using specific PCR primers and probes. The translocation specific primers bind to exons in the fusion gene enabling amplification of the region containing clinically relevant breakpoint, and the amplicons are

detected by the translocation specific probes (shown in **Figure 1**). Detection of the GUSB gene is an internal control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.

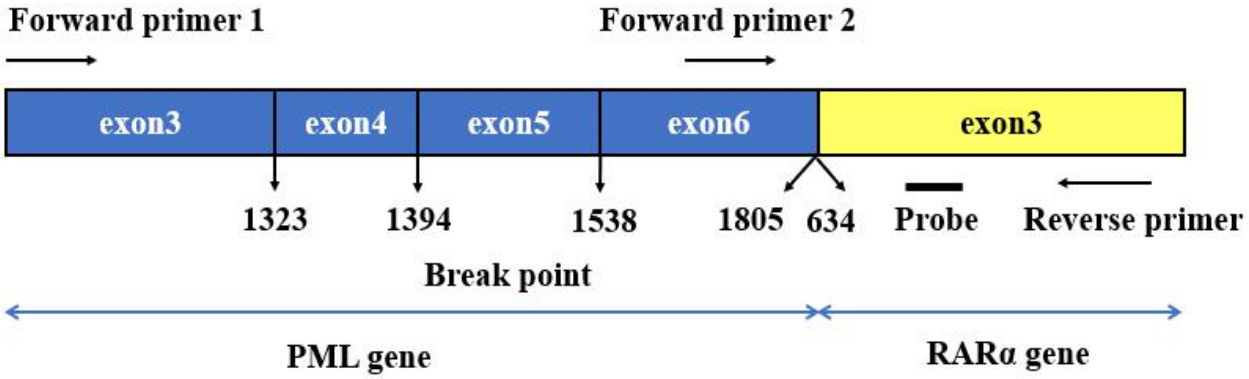


Figure 1. Translocation specific primers and probes are designed to detect multiple clinically relevant breakpoints of fusion genes. In this example, primers and probe for detection of t (15; 17) fusion gene transcript PML-RARα are shown. Exons are numbered for the fusion genes PML and RARα. The three splice variants (S, L, V) of PML-RARα are amplified with two forward primers and one reverse primer, then the amplicons are detected by the probe designed in the exon3 of RARα gene.

3. REAGENTS AND MATERIALS SUPPLIED

LEUKEMIA Fusion Genes (Q51) Screening Kit contains reagents for 20 tests, components are tabulated as **Table 2**.

Table 2. Reagents and Materials in the Kit

Component	Volume (μL)	Description
RT Mix	297	For reverse transcription reaction
RT Enzyme	36	
Q51 PCR Mix A~L	436	For qPCR reaction
LF Polymerase	60	
Q51 Positive control	40	For positive control
LF Negative control	200	For negative control
User manual	1 piece	/

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Real-time thermal cycler with FAM, HEX, ROX and Cy5 channels. Such as Bio-Rad CFX96, Mx3005P/3000P, ABI7500, and SLAN 96s/96p/48p.
- Thermal Heating Block
- Disposable powder-free gloves
- dd H_2O
- Adjustable pipettes and sterile filtered pipette tips
- Vortex mixer
- Desktop centrifuge for 0.1 ml or 0.2 ml optical PCR tubes, 8-tube Strips or 96 plates as well as 1.5 ml microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- 0.1 ml or 0.2 ml optical PCR tubes, 8-tubes strips with Optical Caps or 96 plates with sealing film which match the Bio-Rad CFX96, Mx3005P/3000P, ABI7500 or SLAN 96s/96p/48p.

5. STORAGE CONDITIONS AND SHELF-LIFE

THE kit with all components is stable for 12 months when stored between -25 °C and -18 °C The qPCR reagents including Q51 PCR Mixes A~L must be protected from light to avoid bleaching of the probes. The expiration date of each component is printed on each tube label. Frequent freeze-thaw cycles (>3x) of the kit should be avoided. The kit should be shipped at

low temperature.

6. SAMPLE REQUIREMENTS

COLLECTION OF PRIMARY SAMPLES

Collect 2~3 mL bone marrow or whole blood sample according to routine collection procedure using EDTA or sodium citrate for anticoagulation. DO NOT use heparin due to its inhibition to PCR reaction. Total RNA should be isolated immediately after collection or should be stored at 2-8 ℃ for no more than 24 hours as RNA degrades easily. If not, remove the red blood cells, then add 1 mL of trizol per 5~10×10^6 leukocytes and store at -70 ℃ for no more than one month.

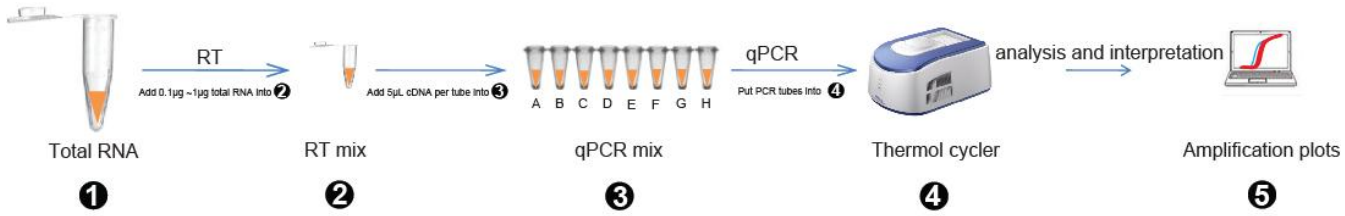
EXTRACTION OF TOTAL RNA

Isolate total RNA from bone marrow or whole blood by an appropriate method, use of trizol is recommended. Since the quality of RNA would affect the test results, it is suggested that the concentration and purity of RNA should be measured with UV spectrophotometer. The ratio of A260/A280 should be within 1.9-2.1, and the ratio of A260/A230 should be above 2.0. In order to avoid RNA degradation, the isolation should be carried out in RNase-free environment, such as a biological safety cabinet or clean bench. Make sure all microcentrifuge tubes, PCR tubes and other consumables are free of RNase. Wear latex gloves, masks or use other protective methods throughout the process to avoid RNase contamination.

MASS CONCENTRATION OF TOTAL RNA USED FOR RT

After isolation, reverse transcription must be done immediately, or the RNA should be stored at -70 °C for no more than 3 months. For accurate detection, the recommended mass concentration of total RNA should be between 20 ng/μL and 200 ng/μL. Before reverse transcription, it is suggested that the integrity of RNA should be evaluated if the laboratory conditions permit. Agarose or denaturing agarose gel electrophoresis is recommended to assess RNA integrity. A 28s rRNA/18s rRNA ratio of 2 means good integrity. If not, the Cq values of Cy5 from PCR mix A~L for each total RNA will be above 25 cycles.

7. TEST PROCEDURE



❶ Add 5 μL (0.1 μg~1 μg) total RNA into RT mix with a total volume of 20 μL

❷ cDNA is synthesized and diluted with 60 μL ddH_2O

❸ 5 μL of cDNA mixes are added into each PCR tubes with qPCR mixes A~L

❹ PCR tubes with qPCR mixes (A~L) are put into thermal cycler, start the qPCR program

❺ Amplification plots are analyzed

Figure 2. Test procedure at a glance.

7.1 REVERSE TRANSCRIPTION (RT)

a. **THAW** the RT Mix at room temperature (15~25 °C) and vortex it for 10 seconds. RT Enzyme is no need to be vortexed. Then spin them briefly to collect the reagents at the bottom of the tubes.

b. **PREPARE** RT reaction mix using PCR tubes for 20 μL reaction volume as **Table 3.**

Table 3. RT Reaction Mix Preparation

Reagents	Volume (μL)/reaction
RT Mix	13.5
RT Enzyme	1.5
Total RNA	5

One RT reaction is for one sample use only. RT reaction mixes for multiple samples should be pre-mixed as a master mix with 5 % overage to cover pipetting losses. Spin the PCR tubes of RT reaction mix briefly. The whole preparation process should be completed within 2 hours and the prepared RT reaction mix should be used for the next step within 1 hour.

c. **PLACE** the PCR tubes of RT reaction mix in Thermal Cycler, incubate at 37 °C for 15 minutes for cDNA synthesis, then 85 °C for 5 seconds for RT enzyme inactivation.

d. **DILUTE** the cDNA with 60 μL of ddH_2O, vortex for 10 seconds and spin the PCR tubes briefly for downstream testing.

7.2 Q-PCR

a. **THAW** all the qPCR reagents and LF Negative Control to room temperature (15~25 °C). Vortex Q51 PCR Mixes A~L for 10 seconds, LF Polymerase and LF Negative Control is no need to be vortexed. Then spin all these tubes briefly to collect the reagents at the bottom of the tubes.

b. **PREPARE** master PCR mixes A~L using 1.5 mL microcentrifuge tubes for 20 μL reaction volume as **Table 4**.

Table 4. Master PCR Mix Preparation

Reaction	Reagents	Volume (μL)
PCR Mix A	Q51 PCR Mix A	19.8n*
	LF Polymerase	0.2n
PCR Mix B	Q51 PCR Mix B	19.8n
	LF Polymerase	0.2n
PCR Mix C	Q51 PCR Mix C	19.8n
	LF Polymerase	0.2n
PCR Mix D	Q51 PCR Mix D	19.8n
	LF Polymerase	0.2n
PCR Mix E	Q51 PCR Mix E	19.8n
	LF Polymerase	0.2n
PCR Mix F	Q51 PCR Mix F	19.8n
	LF Polymerase	0.2n
PCR Mix G	Q51 PCR Mix G	19.8n
	LF Polymerase	0.2n
PCR Mix H	Q51 PCR Mix H	19.8n
	LF Polymerase	0.2n
PCR Mix I	Q51 PCR Mix I	19.8n
	LF Polymerase	0.2n
PCR Mix J	Q51 PCR Mix J	19.8n
	LF Polymerase	0.2n
PCR Mix K	Q51 PCR Mix K	19.8n
	LF Polymerase	0.2n
PCR Mix L	Q51 PCR Mix L	19.8n
	LF Polymerase	0.2n

*n equals the number of samples plus 3, for example, if there are x samples need to be tested in this run, n (n=x+3, the "3" is for Q51 Positive Control, LF Negative Control and pipetting errors respectively) tubes of PCR mix should be prepared.

c. **VORTEXING** the 12 tubes of master PCR mix for 10 seconds and spin the tubes briefly.

d. **DISPENSE** 20 µL of each master PCR mix per well into optical PCR tubes, 8-tubes strips or 96 plates.

e. **ADD** 5 µL of each cDNA, positive control and negative control into PCR mix. A Layout Example is shown in **Figure 3** (take 8-tubes strips for example).

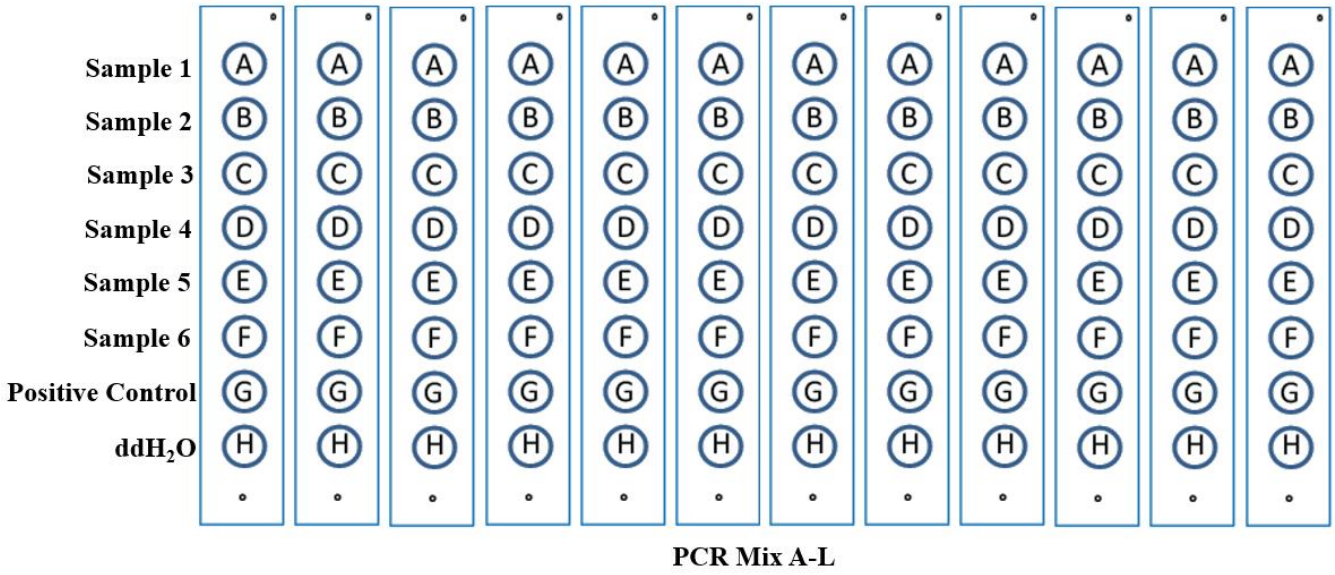


Figure 3. Sample and control distribution in the 8-tubes strips. Six samples were tested by Fusion Genes (Q51) Screening Kit in this example.

f. **SPIN** the PCR tubes briefly to collect the contents at the bottom of the tubes;

g. **PLACE** the PCR tubes, 8-tubes strips or 96 plates in the thermal cycler and run the pre-set program (see **Table 5**):

Table 5. Program of qPCR

Stage	Condition	Cycle number
UNG pre-treatment	50 °C, 2 minutes	1
Initial denaturation	95 °C, 10 minutes	1
Touchdown cycling program	95 °C, 20 seconds 65 °C,1 minute (decreased 1°C per cycle) 72 °C, 1 minute	10
PCR cycling program	95 °C, 20 seconds 56 °C, 32 seconds, collect the fluorescent signal in FAM, HEX, ROX and Cy5 72 °C,1 minute	40

h. **AFTER** the program is finished, put the PCR tubes (closed) into a self-sealing (zip) bag, sealed tightly, and treat as pollutant source.

8. ANALYSIS AND INTERPRETATION

8.1 Cq determination

After the run of qPCR, a threshold line should be carefully set up to allow accurate Cq (shown in **Figure 4**). Note: Threshold line setting should automatically output by the instrument. If the signal occurs too early or fluctuates significantly, the automatic threshold line may be not suitable for data processing. Manually set up baseline threshold according to the original amplification curve and the instruction of thermal cycler.

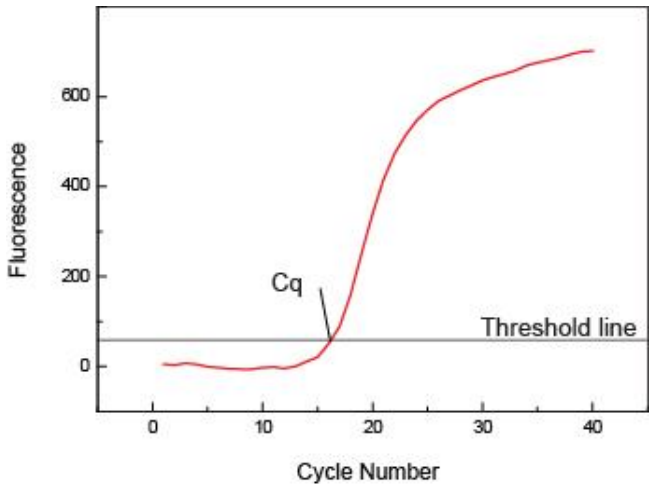


Figure 4. Cq is the intersection between the amplification curve and the threshold line.

8.2 LF Negative Control

No signal should be detected for the LF Negative Control in any channels. If not, the run is invalid and should be repeated.

8.3 Q51 Positive Control

The Cq values of any channels in 12 reactions (mix A~L) in any mix should be less than 25. If not, the run is invalid and should be repeated.

8.4 Samples

a. The Cq values of Cy5 channels in 12 reactions (mix A~L) should be less than 25.

b. Cq values for positive sample signals other than Cy5 should be below 30. Cq values above 30 might be non-specific amplification (shown in **Figure 5**). Repeat the test with fresh RNA, if the second test is positive, the sample is positive for the corresponding fusion gene. Other methods are recommended to be used to confirm the results from positive tests with Ct values above 30.

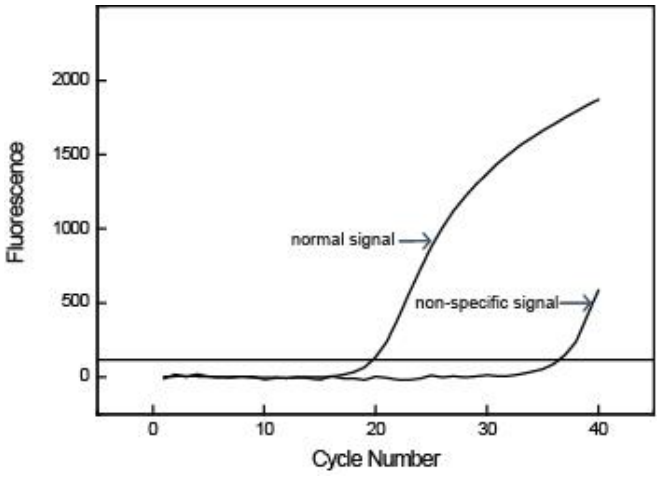


Figure 5. Non-specific signal in sample

c. The fusion gene type for positive samples should be interpreted according to **Table 6**. An example of amplification plots from AML1-ETO positive samples is shown in **Figure 6**.

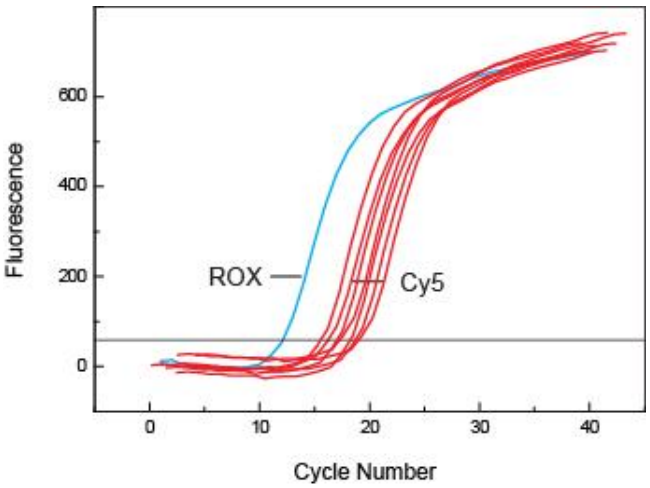


Figure 6. Example of amplification plots from AML1-ETO positive sample by using Leukemia Fusion Genes (Q51) Screening Kit. The Cq of Cy5 (red plots, mix A~L) were below 25 cycles, the Cq of ROX (blue plots, mix A) was below 30 cycles, therefore, the sample was AML1-ETO positive according to table 6.

Table 6. Interpretation Table (A~L represent Q51 PCR Mix A~L)

Channel / Tube	***HEX+Cy5***	***FAM+Cy5***	***ROX+Cy5***	***FAM+ROX+Cy5***	***HEX+FAM+Cy5***	***HEX+ROX+Cy5***
Q51 PCR Mix A	MLL-AF9	PML-RARα	AML1-ETO	/	/	/
Q51 PCR Mix B	MLL-AF4	TEL-AML1	E2A-PBX1	/	FIP1L1-PDGFRA	/
Q51 PCR Mix C	MLL-ENL	BCR-ABL1	SIL-TAL1	/	/	/
Q51 PCR Mix D	MLL-AF10	CBFβ-MYH11	AML1-MDS1/EVI1	/	/	/
Q51 PCR Mix E	EVI1	SET-CAN	E2A-HLF	DEK-CAN	/	/
Q51 PCR Mix F	MLL-SEPT6	TLS-ERG	TEL-PDGFRB	/	/	MLL-ELL
Q51 PCR Mix G	MLL-AF17	NPM-RARα	NPM-MLF	/	PLZF-RARα	MLL-AF1q
Q51 PCR Mix H	MLL-AF1p	TEL-ABL1	AML1-MTG16	AML1-EAP	/	MLL-AF6
Q51 PCR Mix I	BCR-ABL1 p190	BCR-ABL1 p210	NUP98	/	/	/
Q51 PCR Mix J	PML-RARα S	PML-RARα V	CALM-AF10	/	PML-RARα L	/
Q51 PCR Mix K	HOX11	RARα	TEL-JAK2	/	MLL-AFX1	/
Q51 PCR Mix L	ETV6-PDGFRA	HOX11L2	dup MLL	/	NPM-ALK	/

Note

1. NUP98 fusion genes contain NUP98-PMX1, NUP98-HOXD13, NUP98-HOXA9, NUP98-HOXA13, NUP98-HOXC11, NUP98-HOXA11.

2. RARα fusion genes contain STAT5-RARα, NUMA-RARα, FIPIL1-RARα, PRKAR1A-RARα.

3. In addition to the Cy5 channel in 12 reactions (mix A~L), BCR-ABL1 p210 positive samples will also show amplification signals in LF Mix C FAM channel, LF Mix I FAM channel at the same time; BCR-ABL1 p190 positive samples will also show amplification signals in LF Mix C FAM channel and LF Mix I HEX channel at the same time. In addition to the Cy5 signal in the 12 reactions (mix A~L) ,BCR-ABL1 positive samples of other types only show amplification signals in LF Mix C FAM channel.

4. In addition to the Cy5 channel in 12 reactions (mix A~L), PML-RARα L positive samples will also show amplification signals in LF Mix A FAM channel, LF Mix J FAM channel, and HEX channel at the same time; PML-RARα V positive samples will also show amplification signals in LF Mix A FAM channel and LF Mix J FAM channel at the same time; PML-RARα S positive samples will also show amplification signals in LF Mix A FAM channel and LF Mix J HEX channel at the same time.

9. LIMITATION OF THE METHOD

- The kit can only detect 52 common fusion genes, other rare fusion genes are not covered.
- For most of the genes, the kit can only report the types of fusion genes, but cannot differentiate the splice variants.
- The Cq values cannot be used for quantification of the fusion transcripts level.
- The low level of fusion transcripts in samples can result in false negative results because of the kit's limit of detection.

10. PERFORMANCE CHARACTERISTICS

LIMIT OF DETECTION: 1000 copies per reaction.

11. WARNING AND PRECAUTIONS

THE instruction must be followed exactly to get accurate results.

- Do not pool reagents from different kits or lots, and do not use after the expiration date printed on the external box.
- Use aerosol barrier pipette tips.
- Laboratory workbenches, pipettes and other consumables must be cleaned with bleach regularly.
- Opening PCR tubes after amplification should be avoided in order to minimize the risk of contamination.
- Gloves should be worn during the whole operation.
- The test results should not be used as the only reference for treatment planning. Clinicians should make comprehensive
- decision in consideration of patient`s condition, drug indications, therapeutic response and so on.

12. BIBLIOGRAPHY

1. de The H, Chen Z. Acute promyelocytic leukemia: novel insights into the mechanisms of cure. Nat Rev Cancer. 2010;10(11):775-783.

2. Meyer C, Kowarz E, Hofmann J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009; 23(8):1490-1499.

3. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol. 2009;27(35):6041-6051.

4. Rowley JD. Chromosomal translocations: revisited yet again. Blood. 2008;112(6):2183-2189.

5. Frohling S, Dohner H. Chromosomal abnormalities in cancer. N Engl J Med. 2008;359(7):722-734.

6. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood. 2008;112(13):4808-4817.

7. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 2008;111(5):2505-2515.

8. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer. 2007;7(4):233-245.

9. Pallisgaard N, Hokland P, Riishoj DC, et al. Multiplex reverse transcription polymerase chain reaction for simultaneous screening of 29 translocations and chromosomal aberrations in acute leukemia. Blood. 1998;92(2):574-588.

Key to symbols used in the package:

REF Catalogue Number

Manufacturer

Production Date

-18℃
-25℃ Store between-25°C and -18°C

Expiration Date

Do Not Reuse

CAUTION

Consult Instructions For Use

Number of Tests

Xiamen Zeesan Biotech Co., Ltd.
Building 1#, 4F and A area 3F of Building 11#, No.3701 North Xiang'an Road, (Xiang An) Industrial Area, Torch High-Tech Zone, Xiamen City, Fujian Province, 361101, P. R. China
ALERCELL, INC.
5 West Mendenhall Suite 202
Bozeman MT 59715 USA
Tel: (406) 233 6434 or (888) 960-0855
www.alercell.com

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