Leukemia Fusion Genes (Q30) 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 **803101 RUO**

20 Tests per Kit
For Research Use Only

Table of Contents

1. INTENDED USE

LEUKEMIA Fusion Genes (Q30) Screening Kit is a qualitative test for screening of 30 fusion genes resulted from chromosome translocations involved in chronic and acute leukemia. All the 30 fusion genes include more than 155 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 fusion gene detected by the kit allows professionals to be more aware of the patient`s prognosis and provides professionals important insights into the treatment planning.

THE Kit detects the following 30 chromosome translocations (**Table 1**):

Table 1. 30 Chromosome Translocations in the Kit

No.	Translocation	Fusion Gene	No.	Translocation	Fusion Gene
1	t(9;11)(p22;q23)	MLL-AF9	**16**	t(6;9)(p23;q34)	DEK-CAN
2	t(15;17)(q24;q21)	PML-RARα	**17**	t(X;11)(q24;q23)	MLL-SEPT6
3	t(8;21)(q22; q22)	AML1-ETO	**18**	t(16;21)(p11;q22)	TLS-ERG
4	t(4;11)(q21;q23)	MLL-AF4	**19**	t(5;12)(q33;p13)	TEL-PDGFRB
5	t(12;21)(p13;q22)	TEL-AML1	**20**	t(11;19)(q23;p13.1)	MLL-ELL
6	t(1;19)(q23;p13)	E2A-PBX1	**21**	t(11;17)(q23; q21)	MLL-AF17
7	t(11;19)(q23;p13.3)	MLL-ENL	**22**	t(5;17)(q35;q21)	NPM-RARα
8	t(9;22)(q34;q11)	BCR-ABL1	**23**	t(3;5)(q25;q34)	NPM-MLF1
9	del(1)(p32)	SIL-TAL1	**24**	t(11;17)(q23;q21)	PLZF-RARα
10	t(10;11)(p12;q23)	MLL-AF10	**25**	t(1;11)(q21;q23)	MLL-AF1q
11	inv(16)(p13;q22)	CBFβ-MYH11	**26**	t(1;11)(p32;q23)	MLL-AF1P
12	t(3;21)(q26; q22)	AML1-MDS1/EV11	**27**	t(9;12) (q34;p13)	TEL-ABL1
13	del(4)(q12)	FIP1L1-PDGFRA	**28**	t(16;21)(q24;q22)	AML1-MTG16
14	del(9)(q34)	SET-CAN	**29**	t(3;21)(q26; q22)	AML1-EAP
15	t(17;19)(q22;p13)	E2A-HLF	**30**	t(6;11)(q27;q23)	MLL-AF6

2. PRINCIPLE OF THE PROCEDURES

LEUKEMIA Fusion Genes (Q30) 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 8 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 the clinical 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.

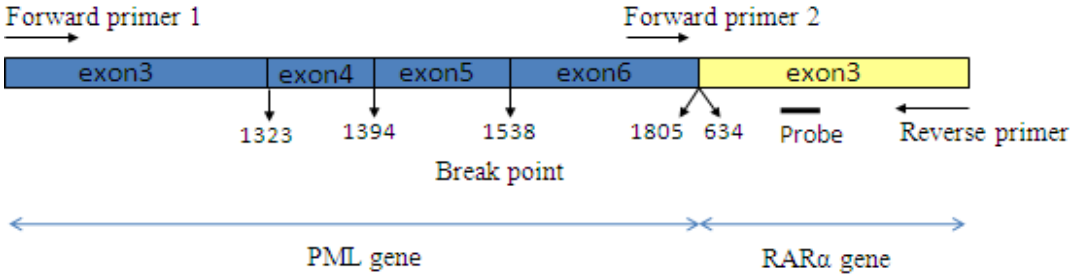


Figure 1. Translocation specific primers and probes are designed to detect multiple clinical relevant breakpoints of fusion genes. In this example, primers and probe for detection of t(15;17) fusion gene transcript PML-RARα are shown. Exons are numbered for the fusion genes PML and RARα. The three splice 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 (Q30) Screening Kit contains reagents for 20 tests, components are tabulated as below (table 2):

Table 2. Reagents and Materials in the Kit

RT Buffer	tube 1	44 μL	
RT Enzyme	tube 2	13 μL	Reverse transcription (RT)
RT Primer	tube 3	44 μL	reagents
DEPC H_2O	tube 4	30 μL	
LF PCR Mix A~H	tube A~H	436 μL	qPCR reagents
LF Polymerase	tube 5	40 μL	
LF Positive control	tube 6	40 μL	for positive control
LF Negative control	tube 7	200 μL	for negative control
User manual	1 piece	/	/

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Real-time thermal cycler with FAM (Abs 495 nm, Em 520 nm), HEX (Abs 535nm, Em 556 nm), ROX (Abs 585 nm, Em 605 nm) and Cy5 (Abs 650 nm, Em 670 nm) detection channels. We recommend 5 types of PCR thermal cycler including Bio-Rad CFX96, Mx3005P/3000P, ABI7500, Light Cycler 96 and SLAN 96s.
- Thermal Heating Block
- Disposable powder-free gloves
- dd H_2O
- Adjustable pipettes and sterile filtered pipette tips
- Vortex mixer
- Desktop centrifuge for 0.2 ml optical PCR tubes, 8-tube Strips or 96 plates as well as 1.5 ml microcentrifuge tubes
- 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, Light Cycler 96 or SLAN 96s.

5. STORAGE CONDITIONS AND SHELF-LIFE

THE kit with all components is stable for 12 months when stored within -25 ℃ ~ -18 ℃. The qPCR reagents including LF PCR Mixes A~H 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-thraw cycles (>3x) of the kit should be avoided. The kit should be shipped with sufficient ice packs to keep the low temperature.

6. SAMPLE REQUIREMENTS

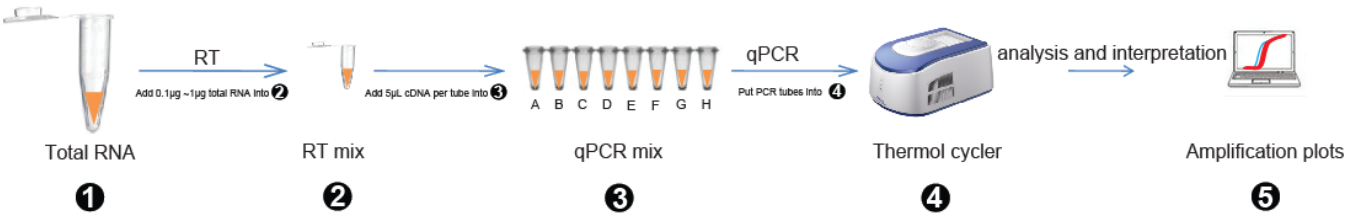
COLLECTION OF PRIMARY SAMPLE: Collect 2~3mL bone marrow or whole blood sample according to routine collection

procedure using EDTA or Sodium citrate for anticoagulation. DO NOT use heparin because of 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 EP 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 ℃ for no more than 3 month. For accurate detection, the recommended mass concentration of total RNA should be between 20ng/μL and 200ng/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~H for each total RNA will be above 25 cycles.

7. TEST PROCEDURE



❶ Add 5μL (0.1μg~1μg) total RNA into RT mix with a total volume of 10μL;

❷ cDNA is synthesized and diluted with 40μL ddH_2O;

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

❹ PCR tubes with qPCR mixes (A~H) are put into thermal cycler, cDNA is then amplified using the qPCR program;

❺ Amplification plots are analyzed.

Figure 2. Test procedure at a glance.

7.1 REVERSE TRANSCRIPTION (RT)

a. **THAW** the **RT reagents** and **LF Positive Control** at room temperature (15~25 ℃). Vortex **RT Buffer** and **RT Primer** for 10 seconds, and **LF Positive control** for 30 seconds. **RT Enzyme** and **DEPC H_2O** need not to be vortexed, Then spin all these tubes for 5 seconds at 3000 rpm/min to collect the reagents the bottom of the tubes.

b. **INCUBATE** 10μL of **LF Positive control** for 5 minutes at 70 ℃ to release RNA in a new 1.5 ml microcentrifuge tube. Spin the tube at 3000 rpm/min to collect the condensate to the bottom of the tubes.

c. **PREPARE** RT reaction mix using 0.2 mL PCR tubes for 10μL reaction volume as Table 3:

Table 3. RT Reaction Mix Preparation

Reagents	Volume (μL)/reaction
RT Buffer	2
RT Primer	2
RT Enzyme	0.5
Total RNA	5
DEPC H_2O	0.5

One RT reaction is for one sample use only. RT reaction mixes for multiple samples (as well as LF Positive Control) should be pre-mixed as a master mix with 5% overage to cover pipetting losses. Spin the PCR tubes of RT reaction mix for 5 seconds at 3000 rpm/min. 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.

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

e. **DILUTE** the cDNA with 40μL of ddH_2O, vortex for 10 seconds and spin the PCR tubes for 5 seconds at 3000 rpm/min for downstream testing.

7.2 Q-PCR

a. **THAW** all the **qPCR reagents** and **LF Negative Control** to room temperature (15~25 ℃). Vortex **LF PCR Mixes A~H** for 10 seconds, **LF polymerase** and **LF Negative Control** need not to be vortexed, then spin all these tubes for 5 seconds at 3000 rpm/min to collect the reagents the bottom of the tubes.

b. **PREPARE** master PCR mixes A~H using 1.5 mL microcentrifuge tubes for 20μL reaction volume as follows (**Table 4**):

Table 4. Master PCR Mix Preparation

Reaction	Reagents	Volume (μL)
PCR Mix A	LF PCR Mix A	19.8n*
	LF Polymerase	0.2n
PCR Mix B	LF PCR Mix B	19.8n
	LF Polymerase	0.2n
PCR Mix C	LF PCR Mix C	19.8n
	LF Polymerase	0.2n
PCR Mix D	LF PCR Mix D	19.8n
	LF Polymerase	0.2n
PCR Mix E	LF PCR Mix E	19.8n
	LF Polymerase	0.2n
PCR Mix F	LF PCR Mix F	19.8n
	LF Polymerase	0.2n
PCR Mix G	LF PCR Mix G	19.8n
	LF Polymerase	0.2n
PCR Mix H	LF PCR Mix H	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 LF Positive Control, LF Negative Control and pipetting errors respectively) tubes of PCR mix should be prepared.

c. **VORTEXING** the 8 tubes of master PCR mix for 10 seconds and spin the tubes for 5 seconds at 3000 rpm/min.

d. **DISPENSE** 20 μL of each master PCR mix per well into 0.2 ml optical PCR tubes, 8-tubes strips or 96 plates.

e. **ADD** 5 μL of each cDNA templates and positive control as well as negative control into PCR mix according to the Figure 3 below (take 8-tubes strips for example).

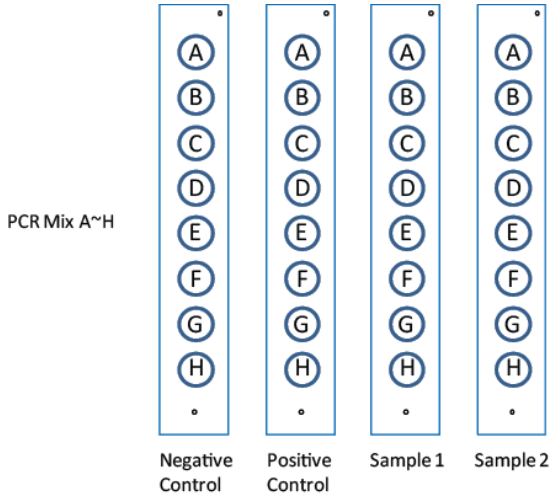


Figure 3. Sample and control distribution in the 8-tubes strips. Two samples were tested by Leukemia Fusion Genes (Q30) Screening Kit in this example.

f. **SPIN** the PCR tubes for 5 seconds at 3,000 rpm/min 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 qPCR Instrument

Stage	Condition	Cycle number
UNG pre-treatment	50 ℃,2 minutes	1
Initial denaturation	95 ℃,3 minutes	1
Touchdown cycling program	95 ℃,20 seconds 65 ℃,1 minute (decreased 1 ℃ per cycle) 72 ℃,1 minute	10
PCR cycling program	95 ℃,20 seconds 56 ℃, 32 seconds, collect the fluorescent signal in FAM, HEX, ROX and Cy5 72 ℃,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 VALUE 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 automatical 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 instead.

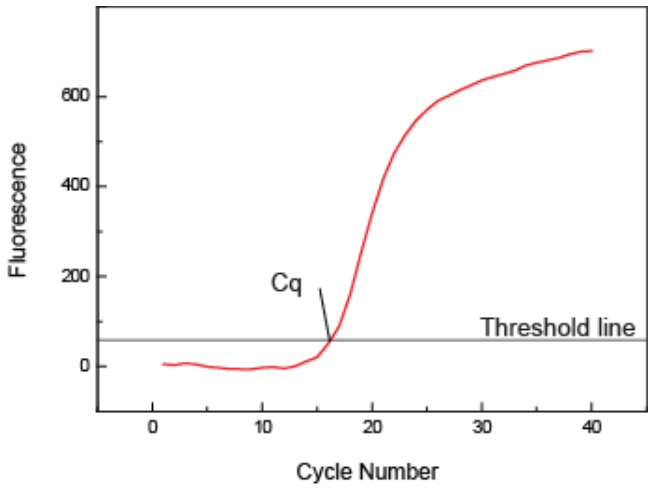


Figure 4. Cq is the intersection between the amplification curve and the threshold line.

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 **LF Positive Control**: The Cq values of Cy5 channels in 8 reactions (mix A~H) as well as FAM channel in mix C should be within 16~20cycles. If not, the run is invalid and should be repeated.

8.4 **Samples**:

a) The Cq values of Cy5 channels in 8 reactions (mix A~H) 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 diagnostic techniques are highly recommended to confirm the results from positive tests with Ct values above 30.

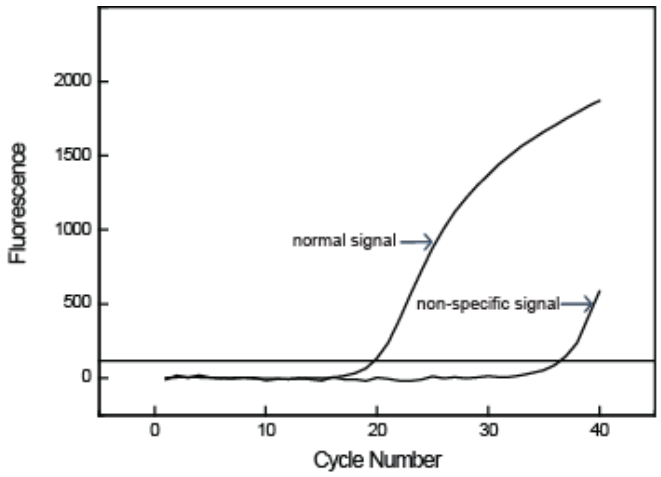


Figure 5. Non-specific signal in sample

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**.

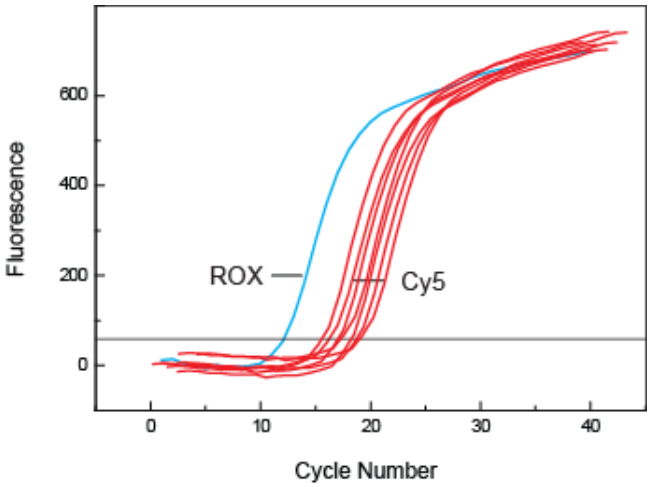


Figure 6. Example of amplification plots from AML1-ETO positive sample by using Leukemia Fusion Genes (Q30) Screening Kit. The Cq of Cy5 (red plots, mix A~H) were below 25cycles, the Cq of ROX (blue plots, mix A) was below 30cycles, therefore, the sample was AML1-ETO positive according to table 6.

Table 6. Interpretation Table(A~H represent LF PCR Mix A~H)

Fluorescence Signals In 8 reaction	Results	Fluorescence Signals In 8 reaction	Results
A :*HEX+Cy5* ; B ~H: *Cy5*	MLL-AF9	F: ***HEX+Cy5*** ; A~E,G,H: ***Cy5***	MLL-SEPT6
A :*FAM+Cy5* ; B ~H: *Cy5*	PML-RARα	F: ***ROX+Cy5*** ; A~E,G,H: ***Cy5***	TEL-PDGFRB
A :*ROX+Cy5* ; B ~H: *Cy5*	AML1-ETO	F: ***FAM+Cy5*** ; A~E,G,H: ***Cy5***	TLS-ERG
B:*HEX+Cy5*;A,C ~H:*Cy5*	MLL-AF4	F: ***HEX+ROX+Cy5*** ; A~E,G,H: ***Cy5***	MLL-ELL
B:*FAM+Cy5*;A,C ~H:*Cy5*	TEL-AML1	G: ***HEX+Cy5*** ; A~F,H: ***Cy5***	MLL-AF17
B:*ROX+Cy5*;A,C ~H:*Cy5*	E2A-PBX1	G:***ROX+Cy5*** ; A~F,H: ***Cy5***	NPM1-MLF1
C:*HEX+Cy5*;A,B,D ~H:*Cy5*	MLL-ENL	G: ***FAM+Cy5*** ; A~F,H: ***Cy5***	NPM1-RARα
C:*FAM+Cy5*;A,B,D ~H:*Cy5*	BCR-ABL1	G: ***HEX+ROX+Cy5*** ; A~F,H: ***Cy5***	MLL-AF1q
C:*ROX+Cy5*;A,B,D ~H:*Cy5*	SIL-TAL1	G: ***HEX+FAM+Cy5*** ; A~F,H: ***Cy5***	PLZF-RARα
D:*HEX+Cy5*;A~C,E~H:*Cy5*	MLL-AF10	H: ***HEX+Cy5*** ; A~G: ***Cy5***	MLL-AF1p
D:*FAM+Cy5*;A~C,E~H:*Cy5*	CBFβ-MYH11	H: ***ROX+Cy5*** ; A~G: ***Cy5***	AML1-MTG16
D:*ROX+Cy5*;A~C,E~H:*Cy5*	AML1-MDS1/EVI1	H: ***FAM+ Cy5*** ; A~G: ***Cy5***	TEL-ABL1
E: *HEX+Cy5* ; A~D,F~H: *Cy5*	FIP1L1-PDGFRA	H: ***HEX+ROX+Cy5*** ; A~G: ***Cy5***	MLL-AF6
E: *ROX+Cy5* ; A~D,F~H: *Cy5*	E2A-HLF	H: ***ROX+FAM+Cy5*** ; A~G: ***Cy5***	AML1-EAP
E: *FAM+Cy5* ; A~D,F~H: *Cy5*	SET-CAN		
E: *ROX+FAM+Cy5* ; A~D,F~H: *Cy5*	DEK-CAN		

9. LIMITATION OF THE METHOD

1) The kit can only detect 30 common fusion genes, other rare fusion genes are not covered.
2) The kit can only report the types of fusion genes, but cannot differentiate the splice variants.
3) The Cq values cannot be used for quantification of the fusion transcripts level.
4) 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

DETECTION sensitivity: 100 copies/reaction.

11. WARNING AND PRECAUTIONS

THE instruction must be followed exactly to get accurate results.

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

12. BIBLIOGRAPHY

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Key to symbols used in the package:

REF Catalogue Number

For Research Use Only

Manufacturer

Production Date

-18℃
-25℃ Store between-25 ℃ and -18 ℃

Expiration Date

Do Not Reuse

CAUTION

Consult Instructions For Use

Number of Tests

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