

Leukemia Gene JAK2 V617F Mutation Real Time RT-PCR Kit User Manual

LT017920RT50

For use with ABI Prism®7000/7300/7500/7900/Step One Plus; iCycler iQ™4/iQ™5; Smart Cycler II;Bio-Rad CFX 96;Rotor Gene™ 6000; Mx3000P/3005P;M-J-Option2/Chromo4; LightCycler®480 Instrument

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1. Intended Use

Leukemia Gene JAK2 V617F Mutation Real Time RT-PCR Kit is used for the detection of Leukemia Gene JAK2 V617F Mutation in leukocyte by using real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

The JAK2 gene was first cloned in 1989 and is a member of a family of four Janus kinases 1, 2 and 3 and tyrosine kinase 2. JAK2 V617F is found in the majority of patients with Ph- myeloproliferative neoplasms (MPNs) and chronic neutrophilic leukemia (CNL). However, it has also been found in many other hematological diseases.

Leukemia Gene JAK2 V617F Mutation Real Time RT-PCR Kit contains a specific ready-to-use system for the detection of the leukemia gene JAK2 V617F mutation using RT-PCR (Reverse Transcription Polymerase Chain Reaction) in the real-time PCR system. The reaction is done in one step real time RT-PCR. The first step is a reverse transcription (RT), during which the mutant gene is transcribed into cDNA. Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of PCR (polymerase chain reaction). Fluorescence is emitted and measured by the real time systems optical unit during the PCR. The detection of amplified fragment is performed in fluorimeter channel FAM with the fluorescent quencher BHQ1. An external positive control (1x10⁷ copies/ml) supplied, allows the determination of the gene load. For further information, please refer to section 9.2 Quantitation.

4. Kit Contents

Ref.	Type of reagent	Presentation	50rxns
1	JAK2-V617F Super Mix	1 vial, 480µl	x2
2	RT-PCR Enzyme Mix	1 vial, 28µl	x2
3	Molecular Grade Water	1 vial, 400µl	x2
4	JAK2-V617F Positive Control (1x10 ⁷ copies/ml)	1 vial, 30µl	x2

Analysis sensitivity: 1x10³ copies/ml; LOQ: 2x10³~1x10⁸ copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the RNA extraction kit recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "epENDOR" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- RNA extraction kit
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.

- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1.1 Leukocytes separation

You can use commercial erythrocytes lysis buffer to remove the erythrocytes from blood samples. Please refer to the specific instructions for erythrocytes lysis buffer.

Attention: Do not use the lymphocytes separation media to obtain leukocytes.

The leukocytes precipitate obtained can be directly used for RNA extraction, and it can also be dissolved in RNA extraction reagents (such as Trizol, RLT buffer) for long-time storage at -80°C. It's strongly recommended not to store the leukocytes precipitate without any RNA extraction reagent.

9.1.2 RNA extraction from leukocytes

RNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kits based on the yield. For the RNA extraction, please comply with the manufacturer's instructions. The recommended extraction kit is as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
Qiagen RNeasy Mini Kit	74106	QIAGEN

e.g. RNA extraction with Trizol

- Add 1 ml Trizol into the leukocytes, and pipet up and down several times to make cells fully dissolved; (Increase the volume of Trizol proportionately if leukocytes are more than 5x10⁶ cells).
- Add 0.2 ml chloroform and shake the tube by vortex for 15 sec at least;
- incubate the tube at room temperature for 2-3 min;
- Centrifuge the sample at 13,000 rpm for 15 min at 4°C.
- The following procedures should be operated on ice box;
- Transfer the aqueous phase above (approximately 0.4-0.6 ml) into a new 1.5 ml centrifuge tube, avoiding disturbing any of the white interphase.
- Add pre-cooled isopropanol into the aqueous phase, and mix by pipetting up and down for 10 times.
- Incubate for 1 hour at -20°C;
- Centrifuge at 13,000 rpm for 15 min at 4°C, and carefully remove the supernatant from the tube;
- Add pre-cooled 75% ethanol and gently pipet RNA pellet;
- Centrifuge the sample at 13,000 rpm for 15 min at 4°C, and carefully remove the supernatant from the tube avoiding disturbing the RNA pellet;
- Add 40 µl DEPC-H₂O to the RNA pellet after Air drying for 5-10 min, then shake gently.
- Centrifuge instantaneously, and incubate for 10 min at room temperature to make RNA fully dissolved.

Extracted RNA can be used for following PCR reactions immediately, or stored at -80°C for long time.

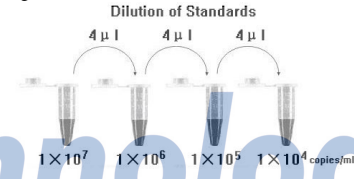
9.2 Quantitation

The kit can be used for quantitative or qualitative real-time RT-PCR. A positive control defined as 1x10⁷ copies/ml is supplied in the kit.

For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows. Molecular Grade Water is used for dilution.

Dilution is not needed for performance of qualitative real-time PCR.

Take positive control (1x10⁷ copies/ml) as the starting high standard in the first tube. Respectively pipette 36ul of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.

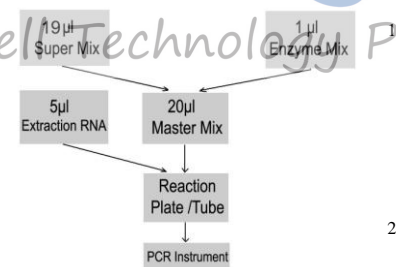
Attention:

- Mix thoroughly before next transfer.
- The positive control (1x10⁷ copies/ml) contains high concentration of the target DNA.

Therefore, be careful during the dilution in order to avoid contamination.

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge. Mix completely then spin down briefly in a centrifuge.

add 5 µl RNA sample, positive and negative controls to different plate/tubes. Immediately close the plate/tubes to avoid contamination.

- Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- Perform the following protocol in the instrument:

45°C for 20min	1 cycle
95°C for 5min	1 cycle
95°C for 15sec, 58°C for 30 sec, 72°C for 45 sec	45 cycles
(Fluorescence measured at 58°C)	

Selection of fluorescence channels	
FAM	Target Nucleic Acid

5) If you use ABI Prism® system, please choose "none" as passive reference and quencher.

10. Threshold setting: just above the maximum level of molecular grade water.

11. Calibration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.

12. Quality control:

Negative control, positive control and QS curve must be performed correctly, otherwise the sample results is invalid.

Channel	Ct value
Control	FAM
Molecular Grade Water	UNDET
Positive Control (qualitative assay)	≤35
QS (quantitative detection)	Correlation coefficient of QS curves ≤ -0.98

13. Data Analysis and Interpretation

The following results are possible:

Ct value	Result Analysis
1	UNDET Below the detection limit or negative
2	≤43 Positive; the sample contains JAK2-V167F mutant gene; and the software displays the quantitative value
3	43~45 Re-test; If it is still 43~45, report as I#