Ultra-Sensitive JAK2 Mutation Detection Kit

User Manual

Catalog Number:	JAK20001-20	JAK20001-50
Size:	20 tests/Kit	50 tests/Kit

Intended Use: For Research Use Only

Doc. No.: 100-JAK20001
Revision: Rev. D

1. PRODUCT INFORMATION

1.1 Background

The Janus kinase 2 gene, also called JAK2, is a non-receptor tyrosine kinase which is involved in cytokine receptor signaling. Activated signaling of JAK2 causes phosphorylation of downstream signal transducers and activators of transcription (STAT) proteins ultimately leading to cell growth and differentiation. Somatic mutation V617F in JAK2 is found in patients with blood related disease, such as polycythemia vera, Thrombocythemia, chronic myelomonocytic leukemia, which may lead to the uncontrolled or abnormal proliferation of blood cells.

1.2 Intended use

Medaysis Ultra-Sensitive JAK2 Mutation Detection Kit is a highly specific and sensitive PCR technique that is able to detect common somatic mutations in the JAK2 gene. Used with Sanger sequencing, it can detect less than 1% (as little as 20 ng to 100 ng of) mutant genes mixed with the wild-type (Table 1). It is designed to amplify JAK2 gene from formalin-fixed paraffin-embedded (FFPE) tissues, fresh or frozen tissues, cell smears, fine needle biopsies (FNA), pleural effusion specimens and plasma samples.
Sample quality assurance for diagnostic tests has not been widely implemented in clinical laboratories.

Table 1. JAK2 mutations detected by the kit:

No	Reagents	Exon	Mutation/ Hot spot	Amino Acid Range
1	JAK2 Exon 14 Primer mix	14	V617	608~621

2. KIT CONTENT

List of components *:

No.	Catalog Number	Name of Components	Volume (µl) JAK20001-20	Volume (µl) JAK20001-50
1	JAK20021	JAK2 Exon 14 PCR primer mix	90	225
2**	JAK20041	JAK2 Exon 14 Seq primer-R	25	60
3	SQC0021	DNA Quality Control Primer Mix	90	225
4	OTH0001	2x PCR Master Mix	450	600
5	OTH0002	Nuclease-Free Water	1000	1000

* Each component contains enough material to test 20 or 50 DNA samples

** R = reverse sequencing primer for Sanger sequencing

3. SHIPPING AND STORAGE

Medaysis JAK2 mutation detection kit is shipped at 4°C and recommends being store at -20°C for long-term storage. When stored under the recommended storage conditions in the original packing, the kit is stable for one year from the date of shipment. Repeated thawing and freezing should be avoided. Non-hazardous. No MSDS required.

4. PRECAUTIONS FOR USE

- Please read the instruction carefully before use.
- The kit is intended for research use only, not for diagnostics purpose.
- Experiments should be performed under proper sterile condition with aseptic techniques.
- All reagents should be thawed thoroughly, mix the components by inverting and centrifuge briefly before use.
- Medaysis Ultra-Sensitive JAK2 mutation detection kit is a PCR-based test to be used by trained laboratory technicians with the appropriate laboratory facilities and equipment.
- Avoid inhalation and ingestion.
- Positive and negative controls should be run simultaneously with all specimens.

5. ADDITIONAL REAGENTS AND INSTRUMENTS REQUIRED

5.1 Reagents

- DNA extraction
- 6 x sample loading buffer
- Agarose

- 1 x TAE buffer
- Novel juice or ethidium bromide
- 100bp DNA ladder standard (Range: 100 – 1000 bp)
- Positive controls: JAK2 Exon 14 mutation control

5.2 Materials

- Sterile, nuclease-free PCR tubes for preparing master mixes
- Adjustable Pipettes for samples preparation
- Disposable sterile pipette tips with filter
- Disposable gloves

5.3 Instruments

- Electrophoresis equipment and power supply
- Sanger Sequencer (our kit is compatible with DNA analyzer ABI3730 and ABI3130)
- The kit has currently been optimized by using ABI Veriti Thermo Cycler. Table 2. List of compatible PCR instruments which has been tested:
- Optimization might be necessary for other instruments. For more information of instrument compatibility, please contact the technical service at Medaysis.

Table 2. PCR instruments

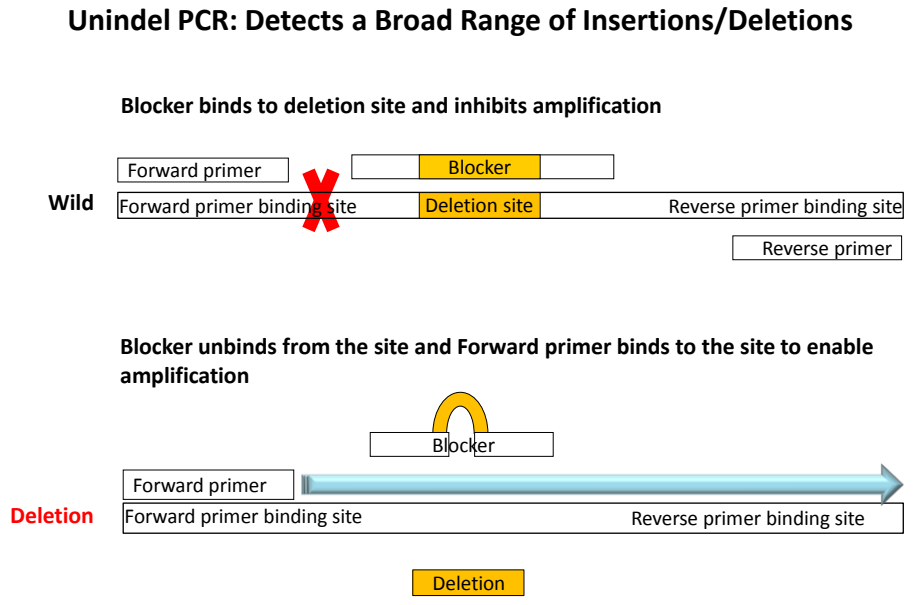
Company	Model
Applied Biosystems *	Veriti
Bio-Rad	T100
Biometra	T-3000

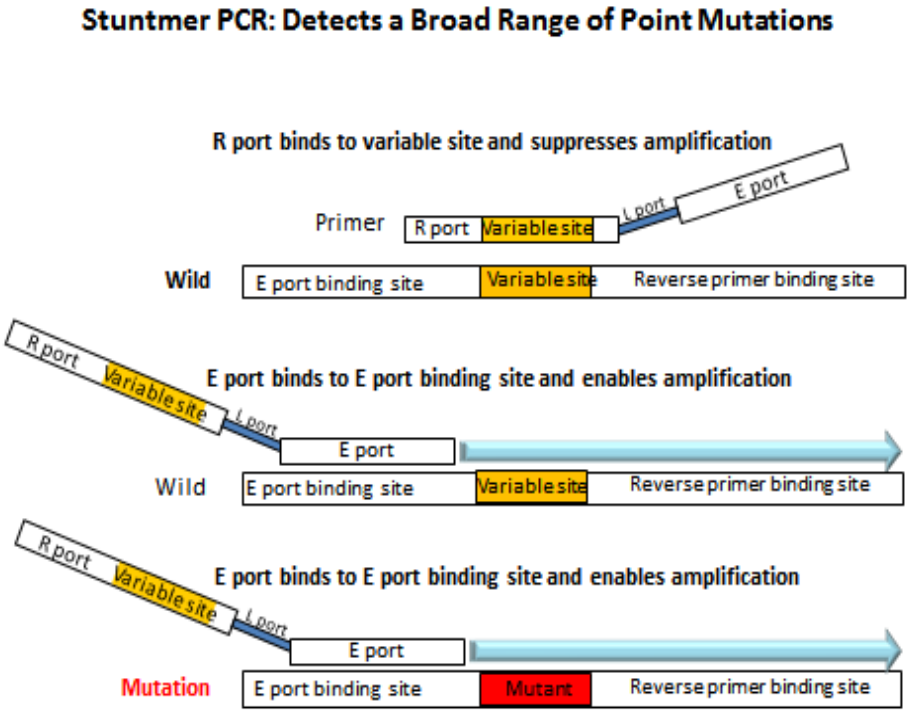
* The assay kit has currently been optimized by using Applied Biosystems Veriti Thermo Cycler.

6. PRODUCT DESCRIPTION AND PRICINPLE

Ultra-Sensitive JAK2 Mutation Detection Kit is a CloDiA™ PCR method using novel and proprietary mutation enrichment technology. CloDiA™ PCR has two types of technique involved - Unindel™ PCR and Stuntmer™ PCR. Unindel™ PCR is designed to detect a broad range of insertions/deletions (universal insertions/deletions) in the target region. The three-primer set consists of forward primer, reverse primer and blocker which inhibits amplification of wild type gene but enables amplification of exonic insertions/ deletions. Stuntmer™ PCR is designed to detect a broad range of point mutations in the target region. The structure of both the forwarder and reverse primer has three ports including R Port, E Port, and L Port to suppress amplification of wild type gene but maximize amplification of mutation type. Stuntmer™ PCR detects a broad range of point mutations in exons 14 of human JAK2 gene. Sanger sequencing can be used to analyze the sequence.

Figure 1. Principle of the Technology.





7. PROTOCOL

To minimize the risk of contamination with foreign DNA, it is recommended that the kits should be conducted in a PCR workstation.

7.1 DNA preparation

Human genomic DNA must be extracted from formalin-fixed paraffin-embedded FFPE tissue, fine needle biopsy or pleural effusion specimens. For FFPE tissue, Medaysis recommends use of Qiagen DNA extraction kit (QIAamp DNA FFPE Tissue Kit, Cat. No. 56404) for genomic DNA extractions. For instructions, refer to the manufacturer's manuals. The kit can be used with DNA extracted with the most common manual and automated extraction methods.
The OD value of genomic DNA extractions should be measured using the spectrophotometer or similar approach. Make sure that OD_{260}/OD_{280} value of sample is between 1.8 and 2.0. Extracted genomic DNA specimens may be stored at -

20°C for long-term storage or refer to the manufacturer's manuals.

For further information regarding the compatibility of the device with different extraction methods please contact the technical support at Medaysis.

7.2 DNA quality control preparation

Numerous molecular detection tests rely on the quality of the genomic DNA specimens. DNA quality control (DNA QC) primer mix is provided to ensure consistent DNA quality.

1. Thaw and centrifuge DNA QC Primer Mix, 2x PCR Master Mix and Nuclease-Free Water at 4°C before use.
2. Prepare one PCR tube and label it as DNA QC.
3. Prepare the PCR Reaction Mixture in the DNA QC tube by adding 10 µl 2x PCR Master Mix, 4 µ l DNA QC Primer Mix and 5 µl Nuclease-Free Water with a total of 19 µl mixture per reaction.
4. Add 1 µl (20~100 ng) DNA specimen into DNA QC tube.
5. Pipette the mixture gently and centrifuge briefly.

Note: If more than one DNA specimens need to be tested, we suggest preparing a reaction mixture containing proportional amount of 2x PCR Master Mix, DNA QC Primer Mix and 5 ul Nuclease-Free Water. Then aliquot 19 ul mixture per reaction to each DNA QC tube and add 1 ul DNA specimen into each tube.

7.3 PCR reaction preparation

1. Thaw and centrifuge all tubes (JAK2 Exon 14 Primer mix, 2x PCR Master Mix and Nuclease-Free Water) at 4°C before use.
2. Prepare PCR tube and label it as S1.
3. Prepare the PCR Reaction Mixture in the S1 tube by adding 10 µl 2x PCR Master Mix, 4 µl JAK2 Exon 14 Primer Mix and 5 µl Nuclease-Free Water with a total of 19µl mixture per reaction.
4. Add 1µl (20~100 ng/µl) DNA specimen into the S1 tube.
5. Pipette the mixture gently and centrifuge briefly

Note: If more than one DNA specimens need to be tested, take the same procedure as the DNA QC preparation. See Table 3 & 4.

Table 3. PCR tube preparation per one reaction:

S1	**DNA QC**	**PC**	**NC**
Exon 14 mixture	DNA Quality Control	Positive control	Negative control

Table 4. Prepare the reaction mixture per one reaction according to the table below:

No.	**Component**	**Volume (µl)**
1	DNA specimen (20~100 ng/µl) or positive or control	**1**
2	Primer Mix	**4**
3	2x PCR Master Mix	**10**
4	Nuclease-Free Water	**5**
	Final Volume	**20**

7.4 PCR thermal cycling condition

Table 5. Follow the PCR protocol exactly when operate PCR instrument

	Temperature (°C)	**Time (min)**	**Cycle(s)**
Stage 1			
Pre-denaturation	95	5	1
Stage 2			

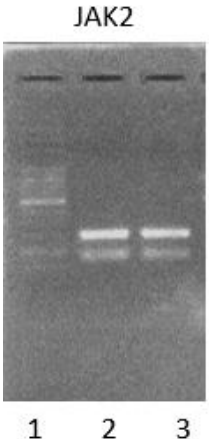
Denaturation	95	0.5	45
Primer Annealing	59	0.5	
Elongation	72	1	
Stage 3			
Extension	72	10	1
Preservation	10	∞	

7.5 Run PCR gel electrophoresis (optional)

Before Sanger sequencing, the PCR products can be examined by the standard agarose gel electrophoresis (2% agarose in 100 ml 1X TAE buffer). The DNA will be visualized by ethidium bromide or novel juice fluorescence.

1. Loading PCR products: mix 5 µl of PCR products with 1 µl of 6X novel juice loading dye and load in the 2% agarose gel.
2. Check the results of JAK2 amplicon (~190 bp) and DNA Quality Control * (both around 190 bp)
3. Perform Sanger sequencing referring to the manufacturer's manuals. Store the rest of PCR products at 4°C.

* DNA Quality Control is used to determine the DNA quality of samples.
Note: For the instructions of DNA sequencer, refer to the manufacturer's manuals.



Lane 1：100 bp DNA Marker

Lane 2：Exon 14 PCR product (194 bp)

Lane 3：DNA Quality Control PCR product (190 bp)

7.6 Recommended Sanger sequencing protocol

Our kit is validated and compatible with DNA analyzer ABI3730. For the instructions of DNA sequencer, refer to the manufacturer's manuals.
For more information of instrument compatibility, please contact the technical service at techsupport@medaysis.com.
Note: PCR products may need to be cleaned up before performing Sanger sequencing.

8. DATA ANALYSIS

PCR products must be sequenced for further analysis. For data analysis, please interpret results refer to the manufacturer's manuals of the software.
Note: To get reasonable interpretation of your results, it is recommended to eliminate baseline "noise" of data.
For the common mutation information, please refer to the following data.

Figure 3. Example of sequence data

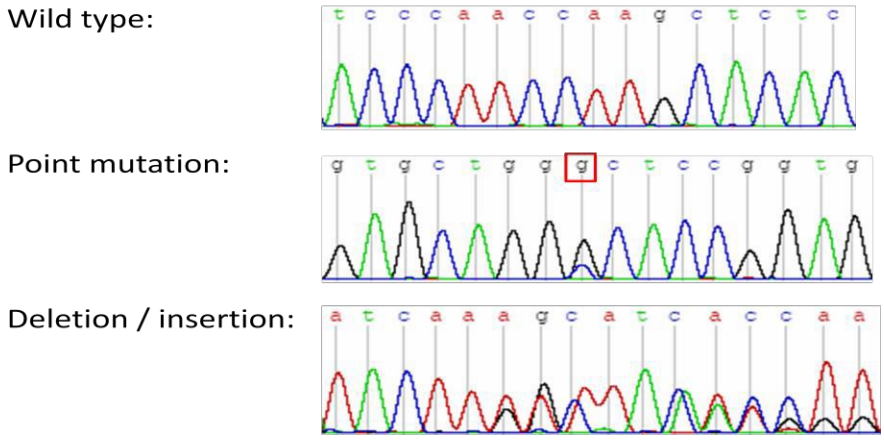


Figure 4. Example of sequence analysis

(A) Harbored a C-To-T transition; (B) Harbored a C-to-T transition and G-to-A transition; (C) Harbored a G-to-A transition.

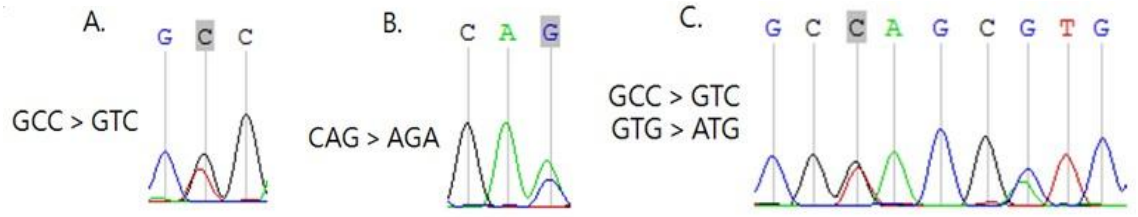
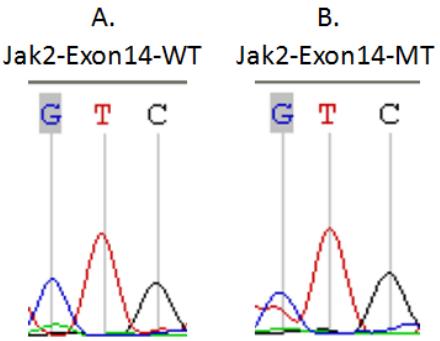


Figure 5. Example of sequence analysis of JAK2 mutation

(A) JAK2 V617 Wild type; (B) JAK2 V617F Harbored a G-to-T transition



9. TROUBLESHOOTING GUIDE

This troubleshooting guide may be helpful in solving similar problems that may arise. If there is any other question, please welcome to contact techsupport@medaysis.com.

Problems	Questions	Suggestions
No amplicon /no band	No PCR products observed on gel electrophoresis.	1. Check the results of DNA Quality Control. If there is no amplicon shown at the size within DNA Quality Control, DNA may be fragmented during inadequate fixation steps. Please check your sample fixation process. 2. Remove presence of inhibitor in reaction in case it exists, and then repeat DNA Quality Control reaction. 3. Inspect temperature calibration on PCR instrument. 4. Check both the storage conditions and the expiration date on the label. Use a new kit if needed.
Non-specific amplification/ multiple products/ wrong size band amplified	How to eliminate the multiple or non-specific PCR products?	1. Conduct kit in PCR workstation to minimize the risk of contamination with foreign DNA. 2. Inspect temperature calibration on PCR instrument. 3. Blocking primer annealing temperature is too low. Increase 2 to 3℃ at annealing step to reduce non-specific binding and amplification.
Equipment variation	Have you checked the discrepancy among different PCR instruments?	Yes. We had done parallel tests on different instruments to make sure our kit compatible with different instrument models including ABI, Biometra, BioRad (Table 2).
Novel mutation	How to verify the novel mutation and confirm the accuracy of the results?	Please check any existed mutations on COSMIC website or do parallel tests with the proven data.

10. REFERENCE

1. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Rumi E, et al. et al. Blood 124:1062-1069. 31, 2014.
2. Unresponsiveness of colon cancer to JAK2(V600E) inhibition through feedback activation of EGFR. Prahallad, A. et al. Nature 483, 100–103, 2012.
3. JAK2 V600E: impli-cations for carcinogenesis and molecular therapy. Cantwell-Dorris ER, et al. Mol Cancer Ther. 10:385–394, 2011.
4. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. James C, et al. Nature. 434:1144-1148, 2005.