

# 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. A

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## 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	JAK20001-50
1	JAK20021	JAK2 Exon 14 PCR primer mix	100	250
2**	JAK20041	JAK2 Exon 14 Seq primer-R	10	25
3	SQC0021	DNA Quality Control Primer Mix	100	250
4	OTH0001	2x PCR Master Mix	1400	3500
5	OTH0002	Nuclease-Free Water	1000	2500

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

\*\* R = reverse 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.

## 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)

## 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. Optimization might be necessary for other instruments. For more information of instrument compatibility, please contact the technical service at Medaysis.

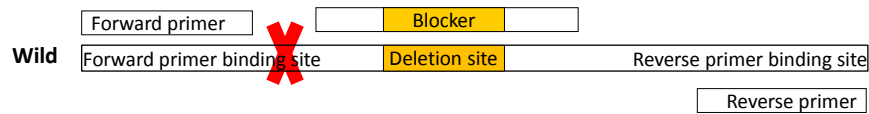
## 6. PRODUCT DESCRIPTION AND PRICINPLE

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.

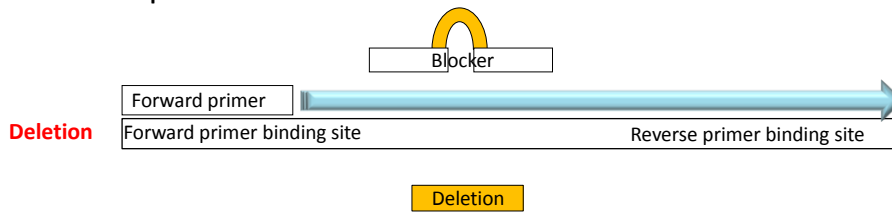
Figure 1. Principle of the Technology.

## Unindel PCR: Detects a Broad Range of Insertions/Deletions

**Blocker binds to deletion site and inhibits amplification**

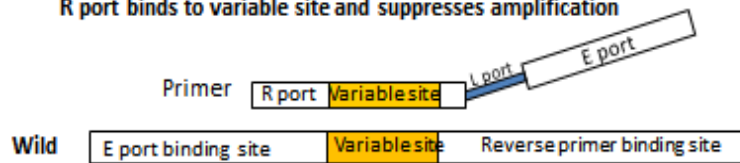


**Blocker unbinds from the site and Forward primer binds to the site to enable amplification**

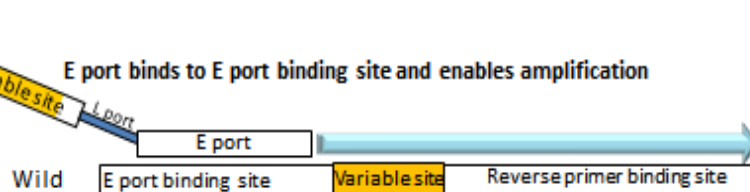


## Stuntmer PCR: Detects a Broad Range of Point Mutations

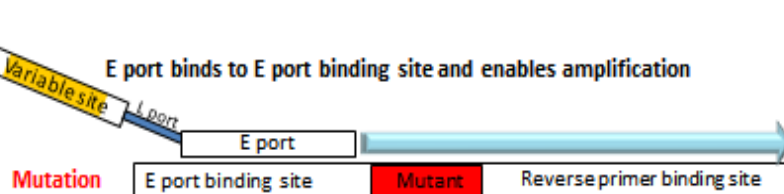
**R port binds to variable site and suppresses amplification**



**E port binds to E port binding site and enables amplification**



**E port binds to E port binding site and enables amplification**



### 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/OD280 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 Sterile H<sub>2</sub>O at 4°C before use.
2. Prepare two PCR tubes and label it as QC and NC.
3. Prepare the PCR Reaction Mixture by adding 10 µl 2x PCR Master Mix, 4.8 µl DNA QC Primer Mix and 4.2 µl Sterile H<sub>2</sub>O with a total of 19 µl mixture per reaction.
4. Add 1 µl (20~100 ng) DNA specimen and 1 µl sterile H<sub>2</sub>O (negative control) into the PCR reaction mixture.
5. Pipette the mixture gently and centrifuge briefly.
6. Mix and Sterile H<sub>2</sub>O. Then aliquot 19 µl mixture per reaction to the PCR tube and add 1µl DNA specimen on each tube.

Note: If more than one DNA specimens need to be tested, we suggest preparing a reaction mix containing appropriate amount of 2x PCR Master Mix, DNA QC Primer.

## 7.3 PCR reaction preparation

1. Thaw and centrifuge all tubes (JAK2 Exon 14 Primer mix, 2x PCR Master Mix and Sterile H<sub>2</sub>O) at 4°C before use.
2. Prepare PCR tube and label it as S1.
3. Prepare separately PCR Reaction Mixture by adding 10µl 2x PCR Master Mix, 4.8µl Primer Mix and 4.2µl Sterile H<sub>2</sub>O with a total of 19µl mixture per reaction for S1
4. Add 1µl (20~100 ng/µl) DNA specimen into the PCR reaction mixture S1
5. Pipette the mixture gently and centrifuge briefly

Note: Same as the preparation of the DNA quality control, if more than one DNA specimens need to be tested, it is recommended to prepare a reaction mix of table3 & 4 (No. 2~4) and aliquot 19µl to each PCR tube.

Table 3. PCR tube preparation per one reaction:

S1	DNA QC	NC
Exon 14 mixture	DNA quality 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) / positive control	1
2	Each primer Mix	4.8
3	2x PCR Master Mix	10
4	Nuclease-Free Water	4.2
	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	10	1
Stage 2			

Denaturation	94	1	45
Blocking Primer Annealing	65	3	
Primer Annealing	57	1	
Elongation	72	1	
<b>Stage 3</b>			
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 100ml 1X TAE buffer). The DNA will be visualized by ethidium bromide or novel juice fluorescence.

1. Loading PCR products: mix 5µl of PCR products with 1 µl of 6X novel juice loading dye and load in the 2% agarose gel.
2. Check the results of each amplicon (~180bp) and sample quality control (100, 200,300, 400 bp)
3. Perform Sanger sequencing referring to the manufacturer’s manuals. Store the rest of PCR products at 4°C.

Note: For the instructions of DNA sequencer, refer to the manufacturer’s manuals.

### 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](mailto:techsupport@medaysis.com).

Note: PCR products may need to be cleaned up before performing Sanger sequencing.

## 8. DATA ANALYSIS

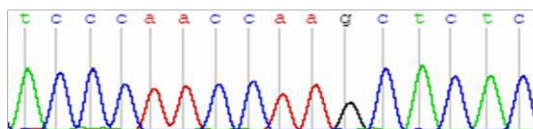
PCR products must be sequenced for further analysis. For data analysis, please interpret results refer to the manufacturer’s manuals of the software.

Note: To get reasonable interpretation of your results, it is recommended to eliminate baseline “noise” of data.

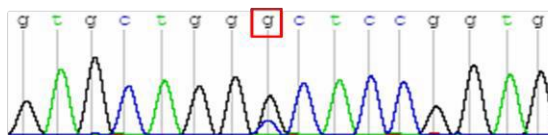
For the common mutation information, please refer to the following data.

Figure 3. Example of sequence data

Wild type:



Point mutation:



Deletion / insertion:

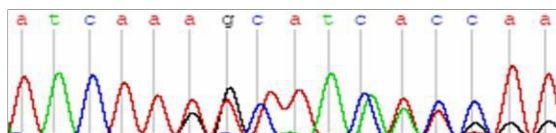


Figure 4. Example of sequence analysis

(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.

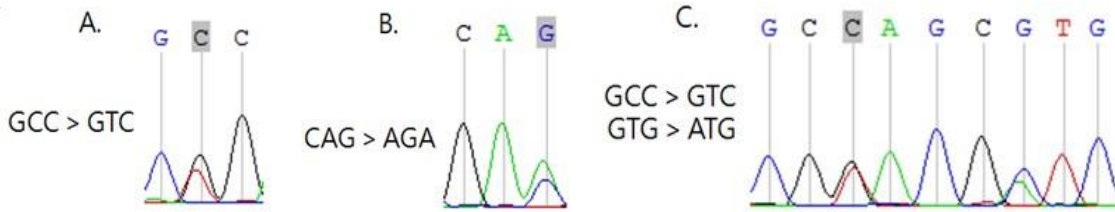
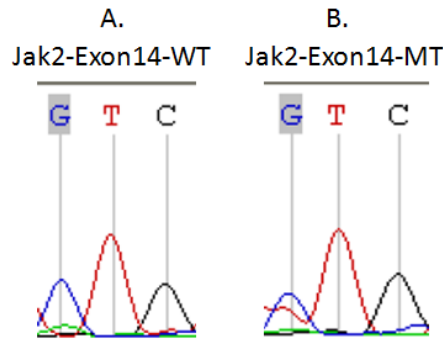


Figure 5. Example of sequence analysis of JAK2 mutation  
(A) JAK2 V617 Wild type; (B) JAK2 V617F Harbored a G-to-T transition



## 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](mailto:techsupport@medaysis.com).

Problems	Questions	Suggestions
No Amplicon /No Band	No PCR products observed on gel electrophoresis.	<ol style="list-style-type: none"> <li>1. Check the results of sample quality control. If there is no amplicon shown at the size of 400bp, DNA may be fragmented during inadequate fixation steps. Please check your sample fixation process.</li> <li>2. Remove presence of inhibitor in reaction in case it exists, and then repeat SQC reaction.</li> <li>3. Inspect temperature calibration on PCR instrument.</li> <li>4. Check both the storage conditions and the expiration date on the label. Use a new kit if needed.</li> </ol>
Non-Specific Amplification/ Multiple Products/ Wrong Size Band Amplified	How to eliminate the multiple or non-specific PCR products?	<ol style="list-style-type: none"> <li>1. Conduct kit in PCR workstation to minimize the risk of contamination with foreign DNA.</li> <li>2. Inspect temperature calibration on PCR instrument.</li> <li>3. Blocking primer annealing temperature is too low. Increase 2 to 3°C at annealing step to reduce non-specific binding and amplification.</li> </ol>
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 (Table2).
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: implications 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.
5. Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. Di Fiore, P.P., et al. Cell, 1987.