Ultra-Sensitive MPL Mutation Detection Kit

User Manual

Catalog Number:	MPL0101-20
Size:	20 tests/Kit
Intended Use:	For Research Use Only
Doc. No.:	100-MPL0101
Revision:	Rev. A

1. PRODUCT INFORMATION

1.1 Background

The MPL gene encodes the thrombopoietin receptor protein, which promotes the cell growth and division. Especially, this thrombopoietin receptor is important for the proliferation of megakaryocytes and the maintenance of hematopoietic stem cells within the bone marrow. Mutations of the MPL gene are usually referred to as Exon 10 W515, which have been associated with essential thrombocythemia and primary (idiopathic) myelofibrosis, leading to the overproduction of abnormal megakaryocytes, an increased number of platelets, as well as many signs and symptoms of the condition. (1-3)

1.2 Intended use

Medaysis Ultra-Sensitive MPL Mutation Detection Kit is a highly specific and sensitive PCR technique that is able to detect common somatic mutations in the MPL 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 MPL gene from formalin-fixed paraffin-embedded (FFPE) tissues, fresh or frozen tissues, cell smears, fine needle biopsies (FNA) or pleural effusion specimens.
Sample quality assurance for diagnostic tests has not been widely implemented in clinical laboratories.

Table 1. MPL mutations detected by the kit:

No	Reagents	Exon	Amino Acid Range
1	MPL Exon 10 Primer mix	10	496~521

2. KIT CONTENT

List of components *:

No.	Catalog Number	Name of Components	Volume (µl)
1	MPL0121	MPL Exon 10 PCR primer mix	90
2*	MPL0141	MPL Exon 10 Seq primer-R	25
3	OTH0001	2x PCR Master Mix	250
4	OTH0002	Nuclease-Free Water	1000

* Each component contains enough material to test 20 DNA samples

* R = reverse sequencing primer for Sanger sequencing

3. SHIPPING AND STORAGE

Medaysis MPL 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 MPL 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

- PCR instrument (Table 2)
- 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.

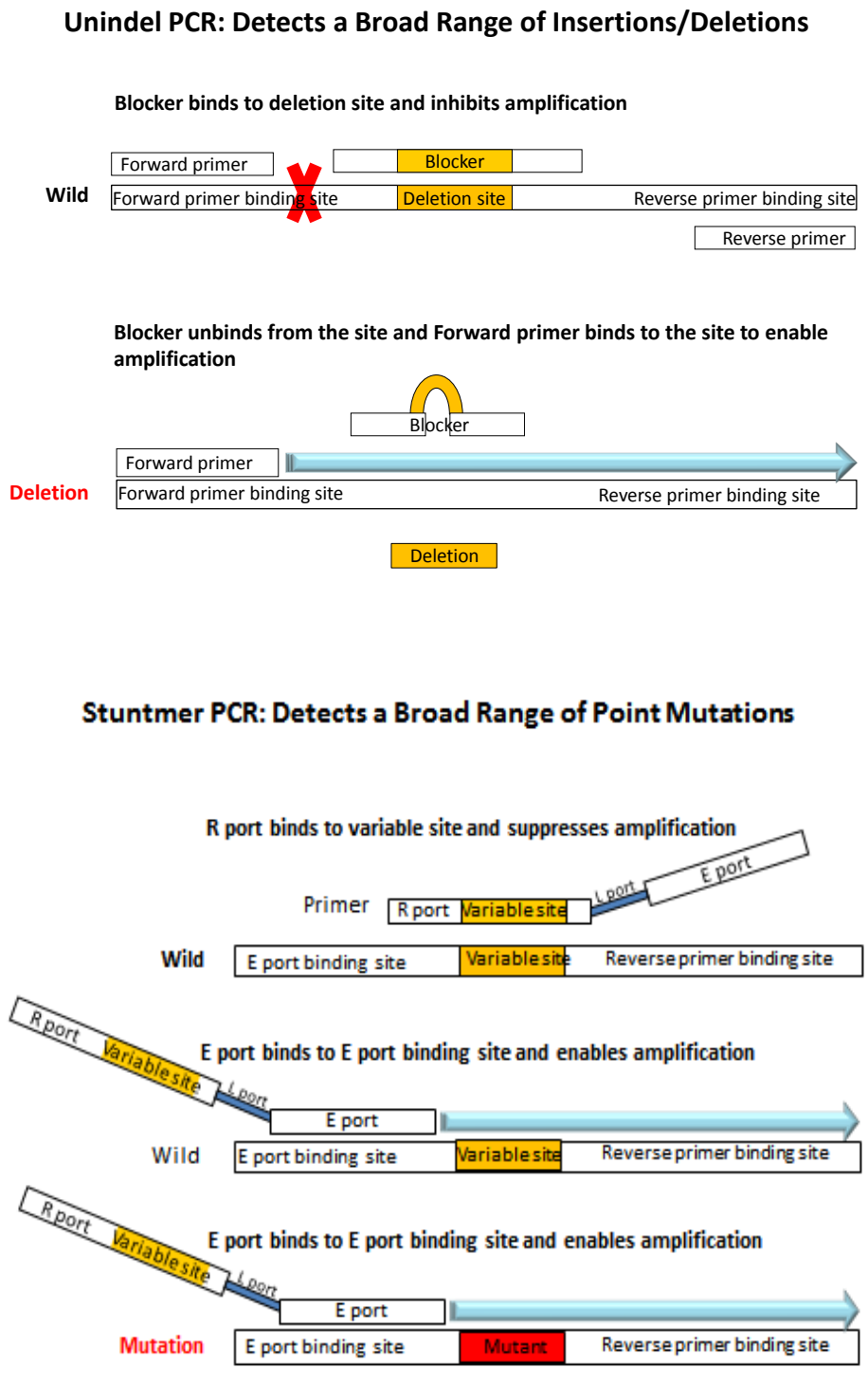
Table 2. PCR instruments

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

6. PRODUCT DESCRIPTION AND PRICINPLE

Ultra-Sensitive MPL 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. 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 clean environment.

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 PCR reaction preparation

1. Thaw and centrifuge all tubes (MPL Exon 10 Primer Mix, 2x PCR Master Mix and Sterile H2O at 4°C before use.
2. Prepare PCR tube and label it as S1.
3. Prepare separately PCR Reaction Mixture by adding 10 µl 2x PCR Master Mix**,** 4 µl Primer Mix and 5 µl Sterile H2O with a total of 19 µl mixture per reaction for S1.
4. Add 1 µl (20~100 ng/ul) 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 Table 3 & 4 (No. 2~4) and aliquot 19µl to each PCR tube.

Table 3. PCR tube preparation per one reaction:

S1	**NC**
Exon 10 mixture	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**
3	2x PCR Master Mix	**10**
4	Nuclease-Free Water	**5**
	Final Volume	**20**

7.3 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	∞	

7.4 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 (~131 bp) (Figure 2)
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.

Figure 2. The amplicons of each PCR product on gel electrophoresis

1：100 bp DNA Marker
2：MPL Exon 10 PCR product (131bp)

Note: All results should be determined based on the sequencing data, not PCR gel electrophoresis.

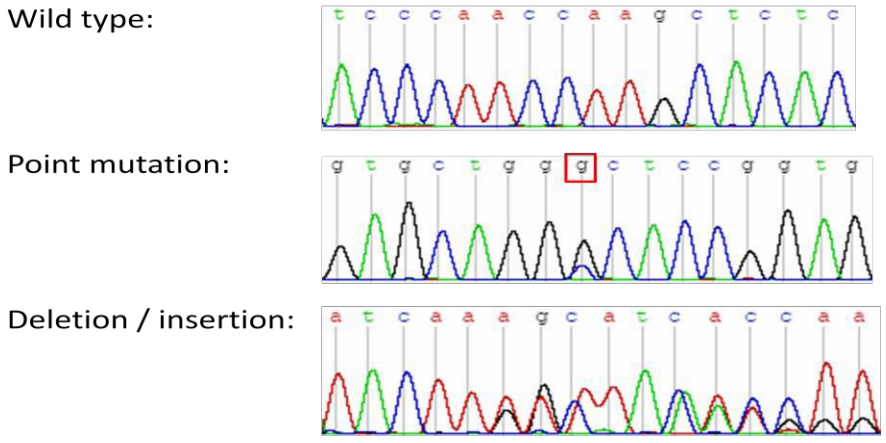
7.5 Recommended Sanger sequencing protocol

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 2. Example of sequence data



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. Remove presence of inhibitor in reaction in case it exists, and then repeat PCR reaction. 2. Test temperature calibration on PCR machine 3. Check both the storage conditions and the expiration date reported on the label. Use a new kit if needed.
Non-Specific Amplification/ Multiple Products/ Wrong Size Band Amplified	How to adjust or eliminate the multiple or non-specific PCR products?	1. Conduct kit in PCR clean environment 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 (Table2).
Novel Mutation	How to verify the novel mutation and confirm the accuracy of the results?	Please check any existing mutations on COSMIC website or do parallel tests with the proven data.

10. REFERENCE

1. Timur Selçuk Akpınar, Veysel Sabri Hançer, Meliha Nalçacı, and Reyhan Diz-Küçükkaya. “MPL W515L/K Mutations in Chronic Myeloproliferative Neoplasms” Turk J Haematol. 2013 Mar; 30(1): 8–12.
2. Chaligné R, Tonetti C, Besancenot R, et al. “New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition.” Leukemia. 2008 Aug;22(8):1557-66.
3. Ronan Chaligné, Chloé James, Carole Tonetti, et al. “Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis” Blood 2007 110:3735-3743.

Orders: customercare@medaysis.com Support: techsupport@medaysis.com Tel: 510-509-3153 www.medaysis.com