Ultra-Sensitive cfDNA ASXL1 Mutation Detection Kit

User Manual

Catalog Number:	ASXL10002-20	ASXL10002-50
Size:	20 tests/Kit	50 tests/Kit

Intended Use: For Research Use Only

Doc. No.: 100-ASXL10002
Revision: Rev. A

© Medaysis Company

1. PRODUCT INFORMATION 3
1.1 Background 3
1.2 Intended use 3
2. KIT CONTENT 3
3. SHIPPING AND STORAGE 4
4. PRECAUTIONS FOR USE 4
5. ADDITIONAL REAGENTS AND INSTRUMENTS REQUIRED 4
5.1 Reagents 4
5.2 Materials 4
5.3 Instruments 4
6. PRODUCT DESCRIPTION AND PRICINPLE 5
7. PROTOCOL 5
7.1 DNA preparation 5
7.2 DNA quality control preparation 5
7.3 PCR reaction preparation 6
7.4 PCR thermal cycling condition 6
7.5 Run PCR gel eletrophoresis (optional) 7
7.6 Recommended Sanger sequencing protocol 7
8. DATA ANALYSIS 8
9. TROUBLESHOOTING GUIDE 9
10. REFERENCE 9

© Medaysis Company

1. PRODUCT INFORMATION

1.1 Background

ASXL1 gene, somatic mutations in the additional sex comb-like 1 (ASXL1), are involved in the regulation or recruitment of the Polycomb-group repressor complex (PRC) and trithorax-group (trxG) activator complex (1). Recently, ASXL1 gene profiling has been studied in various types of diseases, which is also considered as a novel marker which may be a risk factor related with the prevalence and prognostic of myelodysplastic syndromes. In addition, more and more studies indicate that ASXL1 mutations may be associated with a poor outcome (2).

1.2 Intended use

Medaysis Ultra-Sensitive cfDNA ASXL1 Mutation Detection Kit is a highly specific and sensitive PCR technique that is able to detect common somatic mutations in the ASXL1 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 ASXL1 gene in circulating cell-free DNA (cfDNA) from liquid biopsies or blood samples.
Sample quality assurance for diagnostic tests has not been widely implemented in clinical laboratories.

Table 1. cfDNA ASXL1 mutations detected by the Kit:

No	Reagents	Exon	Mutation/Hot spot	Amino Acid Range
1	ASXL1 Exon 12 PCR primer mix (A)	12	G635	619~679
2	ASXL1 Exon 12 PCR primer mix (B)	12	G646	619~679

2. KIT CONTENT

List of components *:

No.	Catalog Number	Name of Components	Volume (µl) ASXL10002-20	Volume (µl) ASXL10002-50
1	ASXL10021	ASXL1 Exon 12 PCR primer mix (A)	100	250
2	ASXL10022	ASXL1 Exon 12 PCR primer mix (B)	100	250
3	ASXL10041	ASXL1 Exon 12 sequencing primer (A)	10	25
4	ASXL10042	ASXL1 Exon12 sequencing primer (B)	10	25
5	SQC0021	DNA Quality Control Primer Mix	100	250
6	OTH0001	2x PCR Master Mix	650	1625
7	OTH0002	Nuclease-Free Water	1000	2500
8	OTH0003	Cell-Free DNA BCT®	20	50

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

3. SHIPPING AND STORAGE

Medaysis cfDNA ASXL1 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 cfDNA ASXL1 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.

© Medaysis Company

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)
- 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

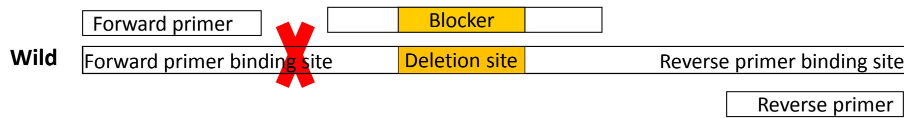
Ultra-Sensitive cfDNA ASXL1 Mutation Detection Kit provides CloDiA™ PCR using novel and proprietary mutation enrichment technology. CloDiA™ PCR has two types of technique involved - Unindel™ PCR and Stuntmer™ PCR. Unindel™ PCR is designed to detect a broad range of insertions/deletions (universal insertions/deletions) in the target region. The three-primer set comprising forward primer, reverse primer and blocker 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. Dual port primer of R Port and E Port is designed for self-competition to preferably amply mutated gene but suppress wild type gene amplification. Stuntmer™ PCR detects a broad range of point mutations in exons 12 of human ASXL1 gene in cfDNA. Sanger sequencing can be used to analyze the sequence.Sanger sequencing can be used to analyze the sequence.

Figure 1. Principle of the Technology

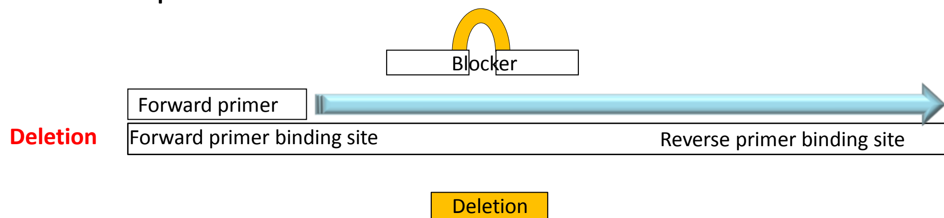
© Medaysis Company

Unindel PCR: Detects a Broad Range of Insertions/Deletions

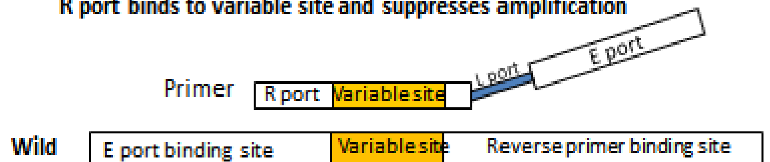
Blocker binds to deletion site and inhibits amplification

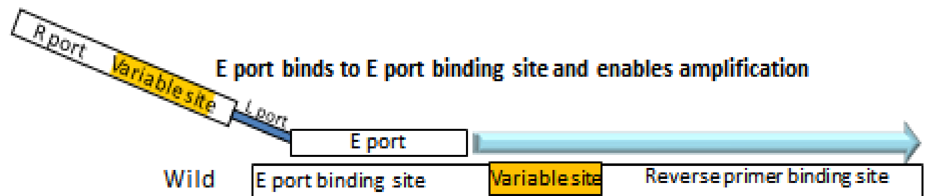


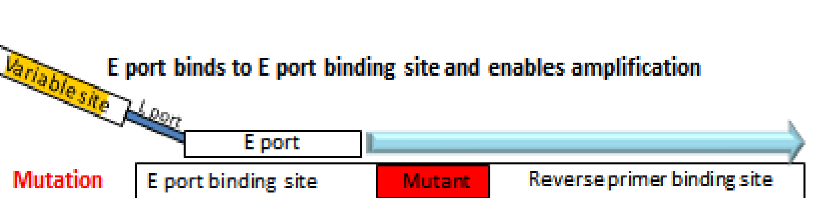
Blocker unbinds from the site and Forward primer binds to the site to enable amplification



Stuntmer PCR: Detects a Broad Range of Point Mutations







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 Plasma sample collection

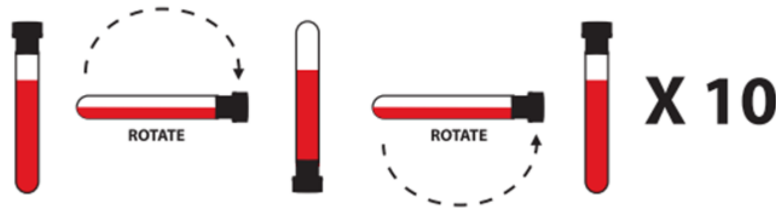
Cell-Free DNA BCT is authorized from Streck and used as 10 mL blood collection tube for stabilization of cell-free plasma DNA. The formaldehyde free preservative in Cell-Free DNA BCT stabilizes white blood cells, preventing the release of cellular genomic DNA and degradation of cfDNA, allowing isolation of high quality cell-free DNA.

© Medaysis Company

Plasma Sample Collection, Processing and Packaging:

1. Collect 10 ml blood samples into Cell-Free DNA BCT tubes until fill tube completely.
2. Mix the tube immediately by gentle inversion 8 to 10 times as Fig.2 below.
3. After collection, transport and store tubes within the recommended temperature range. Note: Blood samples collected in Cell-Free DNA BCT for cfDNA are stable at 6 °C-37 °C up to 14 days. For circulating epithelial cells (tumor cells) in whole blood are stable for up to 4 days at 6 °C-37 °C.
4. Cell-free DNA and cell genomic DNA can be extracted from plasma by using most commercially kits. For instructions, refer to the manufacturer's manuals.

Figure 2. Gently rotate the tube 8 to 10 times



7.2 DNA preparation

Human cell-free DNA and cell genomic DNA can be extracted from plasma by using most commercially manual/automated kits. For instructions, refer to the manufacturer's manuals.
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 techsupport@medaysis.com.

7.3 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 H2O 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 µ l DNA QC Primer Mix and 4.2 µl Sterile H2O with a total of 19 µl mixture per reaction.
4. Add 1 µl (20~100 ng) DNA specimen and 1 µl sterile H2O (negative control) into the PCR reaction mixture.
5. Pipette the mixture gently and centrifuge briefly.

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 Mix and Sterile H2O. Then aliquot 19 µl mixture per reaction to the PCR tube and add 1µl DNA specimen on each tube.

7.4 PCR reaction preparation

1. Thaw and centrifuge all tubes (Exon 12 Primer Mix (A), Exon 12 Primer Mix (B), 2x PCR Master Mix and Sterile H2O) at 4°C before use.
2. Prepare PCR tubes and label them S1 and S2 for each primer mix.
3. Prepare separately PCR Reaction Mixture by adding 10µl 2x PCR Master Mix, 4.8µl Primer Mix and 4.2µl Sterile H2O with a total of 19µl mixture per reaction for S1 and S2.
4. Add 1µl (20~100 ng/µl) DNA specimen into the PCR reaction mixture S1 and S2.
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.

© Medaysis Company

Table 3. PCR tube preparation per one reaction:

S1	S2	DNA QC	NC
Exon 12 mixture (A)	Exon 12 mixture (B)	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**

7.5 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	0:30	45
Primer Annealing	59	0:30	
Elongation	72	1	
Stage 3			
Extension	72	10	1
Preservation	10	∞	

7.6 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 (~250bp).
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.

7.7 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.

© Medaysis Company

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

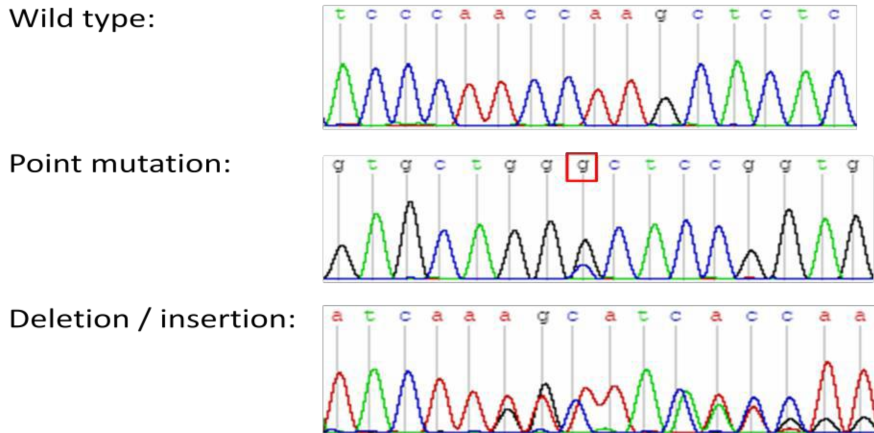
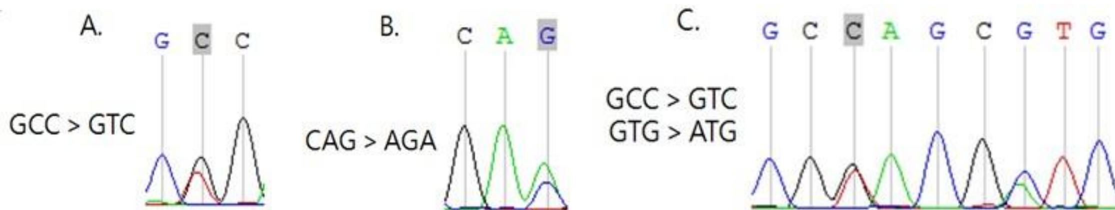


Figure 4. Example of sequence analysis
(A) Harbored a C-To-T transition; (B) Harbored a C-to-T transition and G-to-A transition; (C) Harbored a G-to-A transition.



© Medaysis Company

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 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. 2. Remove presence of inhibitor in reaction in case it exists, and then repeat SQC 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°C 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 existed mutations on COSMIC website or do parallel tests with the proven data.

10. REFERENCE

1. Fisher CL, Randazzo F, Humphries RK, Brock HW. Characterization of Asxl1, a murine homolog of Additional sex combs, and analysis of the Asx-like gene family. Gene. 2006;369:109-18.
2. Chou WC, Huang HH, Hou HA, Chen CY, Tang JL, Yao M, et al. Distinct clinical and biological features of de novo acute myeloid leukemia with additional sex comb-like 1 (ASXL1) mutations. Blood. 2010;116(20):4086-94.

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