Ultra-Sensitive KRAS Mutation Detection Kit

User Manual

Catalog Number:	KRAS0001-20	KRAS0001-50
Size:	20 tests/Kit	50 tests/Kit

Intended Use: For Research Use Only

Doc. No.: 100-KRAS0001
Revision: Rev. A

1. PRODUCT INFORMATION

1.1 Background

KRAS ((Kirsten rat sarcoma-2 viral (v-Ki-ras2) oncogene homolog) gene encodes a small GTPase protein that functions downstream of EGFR-induced cell signaling and participates in the activation of important oncogenic signaling pathways (1). Oncogenic KRAS gene mutations tend to cause constitutive activation, on which the tumor relies to maintain its growth, apoptosis, angiogenesis and metastasis. Therefore, certain studies have been focused on the relation of KRAS gene mutation on different cancer types and others.

1.2 Intended use

Medaysis Ultra-Sensitive KRAS Mutation Detection Kit is a highly specific and sensitive PCR technique that is able to detect common somatic mutations in the KRAS gene (V600). 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 KRAS gene from formalin-fixed paraffin-embedded (FFPE) tissues, fresh or frozen tissues, fine needle biopsies (FNA), pleural effusion specimens, or and plasma samples.
Sample quality assurance for diagnostic tests has not been widely implemented in clinical laboratories.

Table 1. KRAS mutations detected by the kit:

No	Reagents	Exon	Mutation/ Hot spot	Amino Acid Range
1	KRAS Exon 2 PCR primer set	2	G12/G13	3~23
2	KRAS Exon 3 PCR primer set	3	A59/Q61	53~96
3	KRAS Exon 4 PCR primer set (A)	4	K117	107~150
4	KRAS Exon 4 PCR primer set (B)	4	A146	107~150

2. KIT CONTENT

List of components*:

No.	Catalog Number	Name of Components	Volume (µl) KRAS0001-20	Volume (µl) KRAS0001-50
1	KRAS0021	KRAS Exon 2 PCR primer set	100	250
2	KRAS0022	KRAS Exon 3 PCR primer set	100	250
3	KRAS0023	KRAS Exon 4 PCR primer set (A)	100	250
4	KRAS0024	KRAS Exon 4 PCR primer set (B)	100	250
5**	KRAS0041	KRAS Exon 2 Sequencing Primer-R	10	25
6**	KRAS0042	KRAS Exon 3 Sequencing Primer-R	10	25
7**	KRAS0043	KRAS Exon 4 Sequencing Primer-R (A)	10	25
8**	KRAS0044	KRAS Exon 4 Sequencing Primer-F (B)	10	25
9	SQC0021	DNA Quality Control Primer Mix	100	250
10	OTH0001	2x PCR Master Mix	1400	3500
11	OTH0002	Nuclease-Free Water	1000	2500

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

** R = reverse primer for Sanger sequencing; F = forward primer for Sanger sequencing

3. SHIPPING AND STORAGE

Medaysis KRAS mutation detection kit is shipped at 4°C and recommend 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 KRAS 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)
- 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.

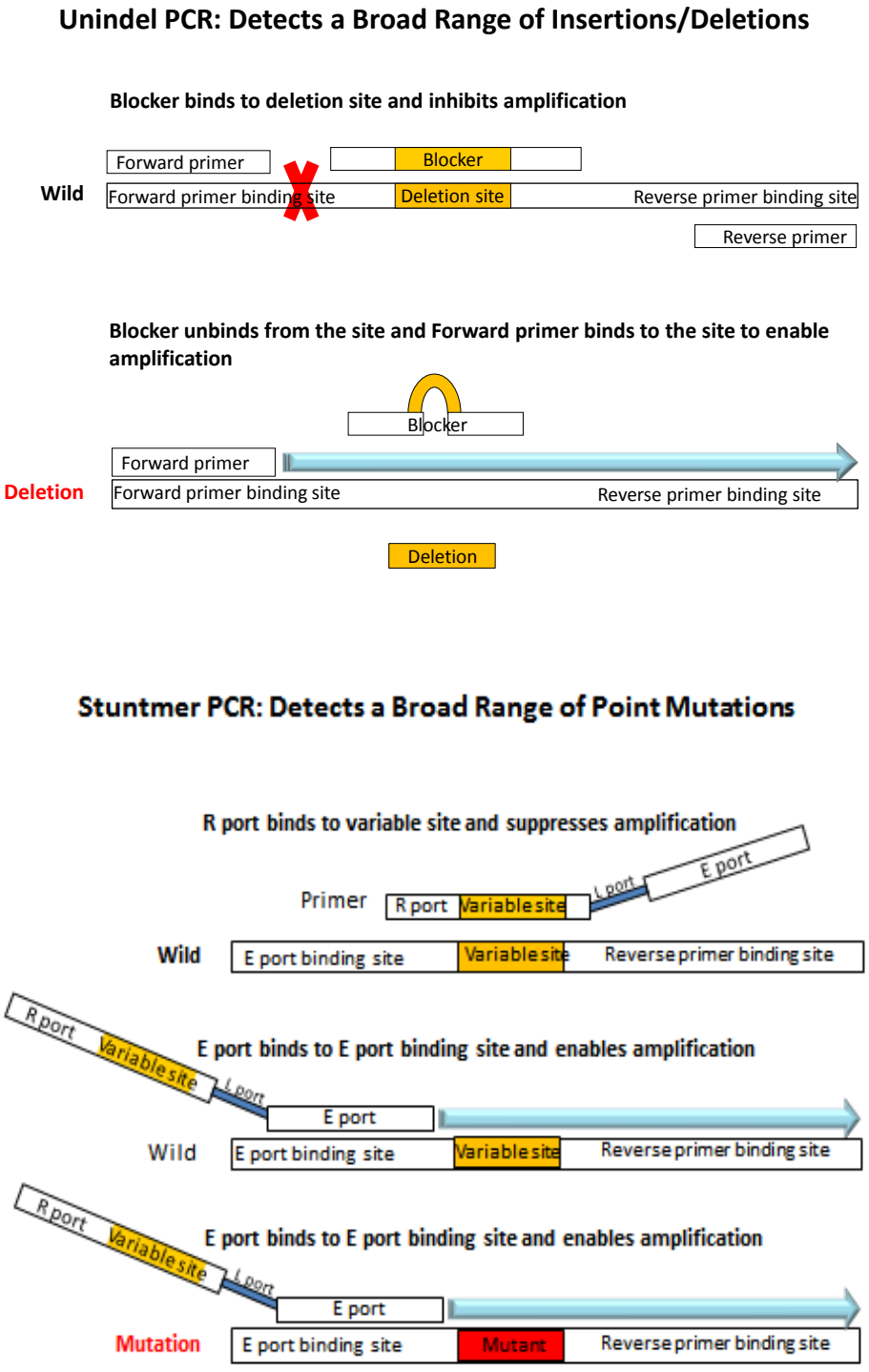
Table 2. PCR instruments

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

6. PRODUCT DESCRIPTION AND PRICINPLE

Ultra-Sensitive KRAS 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 2, 3 and 4 of human KRAS 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 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.
Medaysis KRAS mutation detection 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 techsupport@medaysis.com.

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 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.
6. Mix and Sterile H_2O. 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 primer mix at 4°C before use.
2. Prepare PCR tubes and label them S1, S2, S3, S4 for each primer mix. (Table 3)
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~S4
4. Add 1µl (20~100 ng/µl) DNA specimen into the PCR reaction mixture S1~S4
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 Table4 (No. 2~4) and aliquot 19µl to each PCR tube (S1-S4).

Table 3. PCR tube preparation per one reaction:

S1	S2	S3	S4	DNA QC	NC
Exon2 mixture	Exon3 mixture	Exon 4(A) mixture	Exon 4(B) 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	61	1	
Elongation	72	1	
Stage 3			
Extension	72	10	1
Preservation	10	∞	

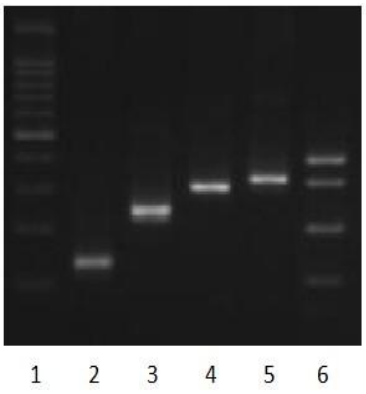
7.5 Run PCR gel electrophoresis (optional)

Before Sanger sequencing, the PCR products can be examined by the standard agarose gel electrophoresis (2% agarose in 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 in 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



Lane 1 : 100bp DNA Marker
Lane 2 : Exon 2 PCR product (~130bp)
Lane 3 : Exon 3 PCR product (~250bp)
Lane 4 : Exon 4A PCR product (~300bp)
Lane 5 : Exon 4B PCR product (~330bp)
Lane 6 : Sample quality control (100, 200, 300, 400, 600bp)

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

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

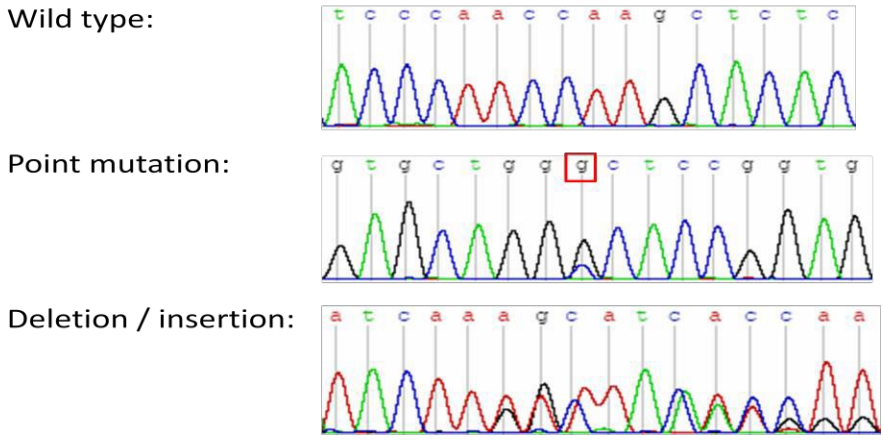


Figure 3. Example of sequence analysis
(A) Harbored a C-To-T transition; (B) Harbored a C-to-T transition and G-to-A transition; (C) Harbored a G-to-A transition.

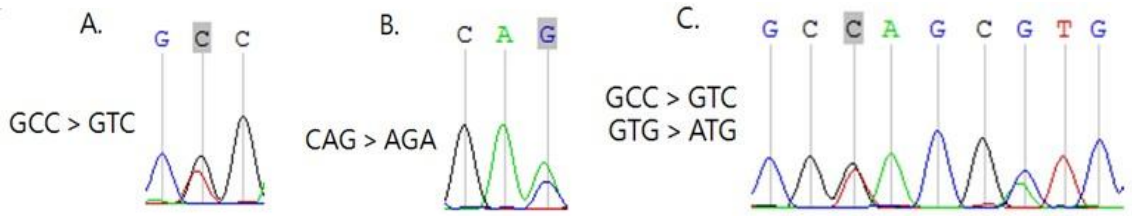
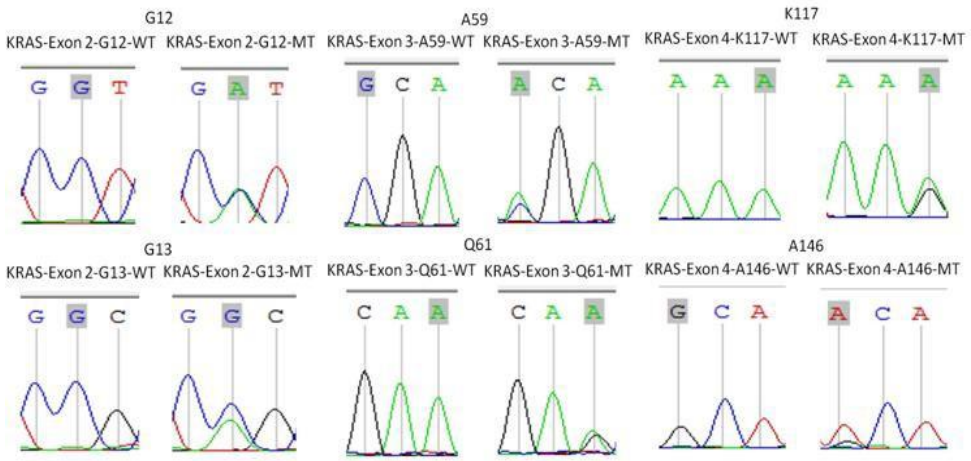


Figure 4. Example of sequence analysis of KRAS mutation.



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℃ 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. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Pai, R., et al., Nat. Med. 2002.
2. Overexpression of the human EGF receptor confers an EGF-dependent transformed phenotype to NIH 3T3 cells. Di Fiore, P.P., et al., Cell, 1987.