Ultra-Sensitive EGFR Mutation Detection Kit

User Manual

Catalog Number:	EGFR0001-20	EGFR0001-50
Size:	20 tests/Kit	50 tests/Kit

Intended Use: For Research Use Only

Doc. No.: 100-EGFR0001
Revision: Rev. B

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1. PRODUCT INFORMATION

1.1 Background

Epidermal growth factor receptor (EGFR) is one of the most frequently amplified, mutated and/or overexpressed genes in human malignancies (1). Inactivation of EGFR kinase with tyrosine kinase inhibitors (TKIs), such as gefitinib (Iressa) and erlotinib (Tarceva), suppresses signal transduction pathways which control tumor cell growth, proliferation, and resistance to apoptosis (2). TKIs therapy has shown a significant symptom benefit and prolongs survival on the treatment of Non-Small Cell Lung Cancer (NSCLC) patients with somatic mutations in the EGFR gene. Therefore, detection of the somatic mutations in the EGFR gene may predict a more accurate response of the cancer patients to TKIs therapy.

1.2 Intended use

Medaysis Ultra-Sensitive EGFR Mutation Detection Kit is a highly specific and sensitive PCR technique that is able to detect common somatic mutations in the EGFR 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 CFDNA EGFR 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. EGFR mutations detected by the kit:

No	Reagents	Exon	Mutation/Hot spot	Amino Acid Range
1	EGFR Exon 18 PCR primer mix	18	G719	688~728
2	EGFR Exon 19 PCR primer mix	19	Exon 19 deletion	739~760
3	EGFR Exon 20 PCR primer mix	20	S768/ Insertion mutation	761~823
4	EGFR Exon 21 PCR primer mix	21	L858 / L861	850~875
5	EGFR Exon 20 T790 PCR primer mix	20	T790	782~823

2. KIT CONTENT

List of components:

No.	Catalog Number	Name of Components	Volume (µl) EGFR0001-20	Volume (µl) EGFR0001-50
1	EGFR0021	EGFR Exon 18 (G719) PCR primer mix	90	225
2	EGFR0022	EGFR Exon 19 del PCR primer mix	90	225
3	EGFR0023	EGFR Exon 20 (S768) PCR primer mix	90	225
4	EGFR0024	EGFR Exon 21 (L858) PCR primer mix	90	225
5	EGFR0025	EGFR Exon 20 T790 PCR primer mix	90	225
6**	EGFR0041	EGFR Exon 18 (G719) sequencing primer-R	25	62.5
7**	EGFR0042	EGFR Exon19 del sequencing primer-R	25	62.5
8**	EGFR0043	EGFR Exon 20 (S768) sequencing primer-R	25	62.5
9**	EGFR0044	EGFR Exon 21(L858) sequencing primer-R	25	62.5
10	EGFR0045	EGFR Exon 20 T790 sequencing primer	25	62.5
11	SQC0021	DNA Quality Control Primer Mix	90	250
12	OTH0001	2x PCR Master Mix	450 x 3 vials	1125 x 3 vials
13	OTH0002	Nuclease-Free Water	1000	2500

**R = Reverse primer for Sanger sequencing

3. SHIPPING AND STORAGE

Medaysis EGFR mutation detection kit is shipped at 4°C and recommends being stored 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 EGFR 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:
    1. EGFR Exon 19 Wild-type Control
    2. EGFR Exon 20 plasmid wild type control
    3. EGFR Exon 21 plasmid wild type
    4. EGFR Exon 19 plasmid deletion

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 techsupport@medaysis.com.

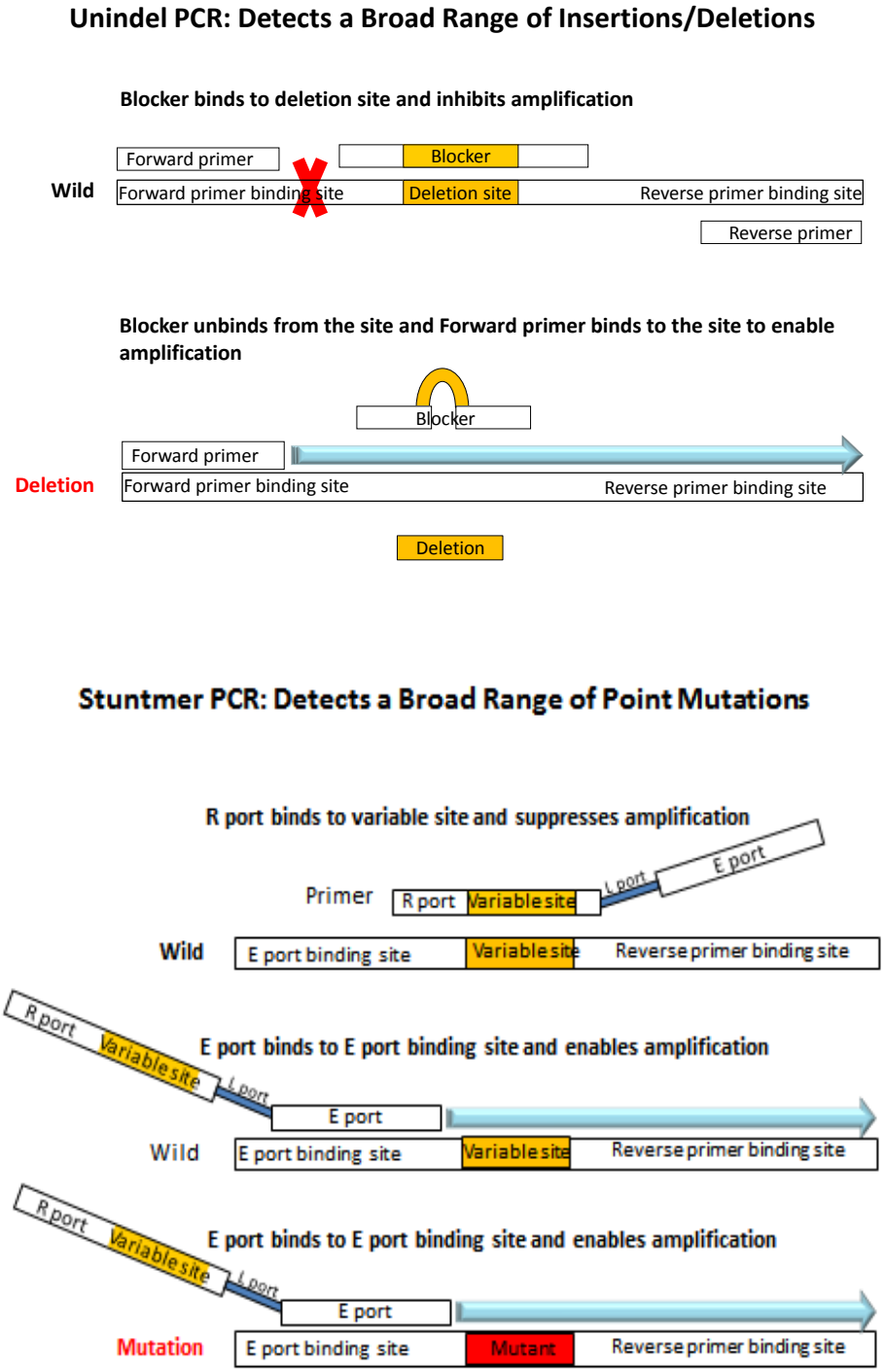
Table 2. PCR instruments

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

6. PRODUCT DESCRIPTION AND PRICINPLE

Ultra-Sensitive EGFR 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. Combination of Unindel™ PCR and Stuntmer™ PCR detects a broad range of insertions/deletions and point mutations in exons 18, 19, 20 and 21 of human EGFR 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. This 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.
3. Prepare the PCR Reaction Mixture by adding 10 µl 2x PCR Master Mix**,** 4 µ l DNA QC Primer Mix and 5 µl Sterile H2O with a total of 19 µl mixture per reaction.
4. Add 1 µl (20~100 ng) DNA into QC mixture as DNA quality control.
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.

7.3 PCR reaction preparation

1. Thaw and centrifuge all tubes (Exon 18/G719, Exon 19 del, Exon 20/S768, Exon 21/L858, T790 each primer set, 2x PCR Master Mix and Sterile H2O) at 4°C before use.
2. Prepare PCR tubes and label them S1, S2, S3, S4, S5 for each primer mix.
3. Prepare separately PCR Reaction Mixture by adding 10µl 2x PCR Master Mix, 4µl Primer Mix and 5µl Sterile H2O with a total of 19µl mixture per reaction for S1~S5.
4. Add 1µl (20~100 ng/µl) DNA specimen into the PCR reaction mixture S1~S5.
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 (S1-S5).

Table 3. PCR tube preparation per one reaction:

S1	S2	S3	S4	S5	DNA QC	PC	NC
Exon 18 (G719) mixture	Exon 19 del mixture	Exon 20 (S768) mixture	Exon 21 (L858) mixture	T790 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) / positive control	**1**
2	Each 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
Blocking Primer Annealing	59	0.5	
Elongation	72	1	
Stage 3			
Extension	72	10	1
Preservation	10	∞	

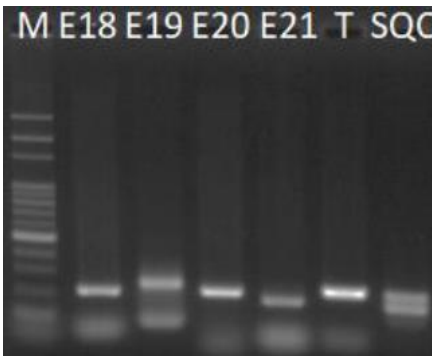
7.5 Run PCR gel electrophoresis (optional)

Before Sanger sequencing, the PCR products can be examined by the standard agarose gel electrophoresis (2% agarose in 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 : 100 bp DNA Marker
Lane 2 : Exon 18 PCR product (~212bp)
Lane 3 : Exon 19 PCR product (blank or ~237bp)
Lane 4 : Exon 20 PCR product (~210bp)
Lane 5 : Exon 21 PCR product (~201bp)
Lane 6 : Exon 20 T790 PCR product (~240bp)
Lane 7 : SQC control (~200 and 220 bp))

Note: It's possible that no amplicon shown at the lane of Exon 19 on agarose gel due to primer design which inhibits wild-type sequence replication during PCR steps. The results should be determined based on the sequencing data.

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

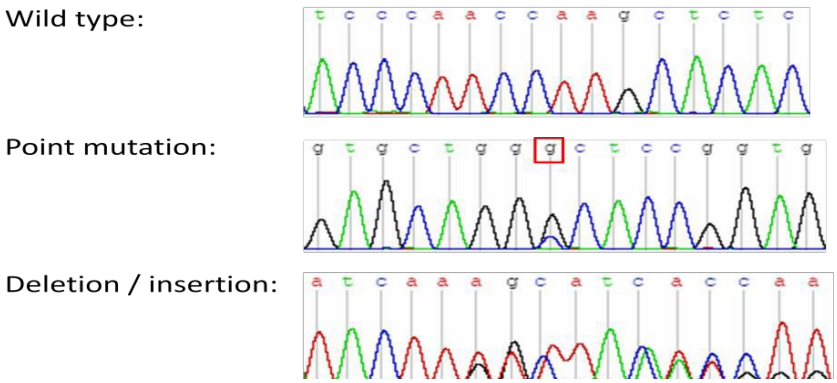
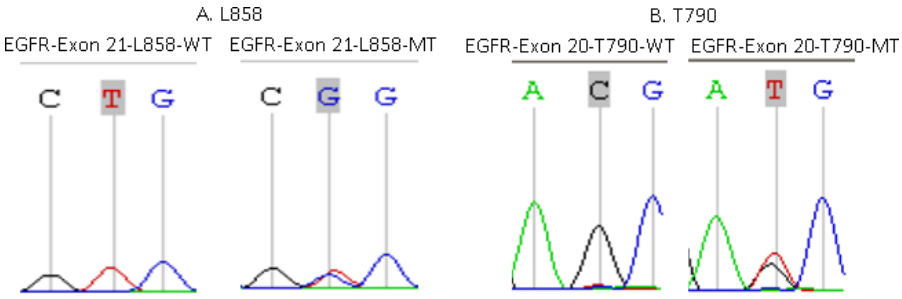


Figure 4. Example of sequence analysis of EGFR mutation.
(A) EGFR Exon21 Harbored a T-to-G transition refers to L858R. (B) EGFR Exon20 T790 Harbored a T-to- G transition refers to T790M.



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.