Detection kit for EGFR Mutations (Fluorescence Probing)

[Production name]

Common name: Detection kit for EGFR Mutations (Fluorescence Probing)

[Production ID]: BD0040

[Packing specifications]: Ten tests

[Intended use]

Detection kit for EGFR Mutations (Fluorescence Probing) is suitable for the detection of EGFR six mutation types in serum, plasma or tissue of non small cell lung cancer patients by adopting fluorescence probing technology. The detection results can reflect the effectiveness of targeted drug such as iressa and tarceva et al, but not use in clinical diagnosis and cure.

[Detection principle]

This kit use TaqMan-MGB probing technology to detect six mutation sites. The 5' end of TaqMan-MGB probe is signed by reporter such as FAM, VIC et al. In addition, the 3' end is non-fluorescence quencher which can't generate fluorescence and reduce background signal greatly. MGB can enhance hybridization between probe and template, and increase Tm of probe. This shorter probe with MGB can reach higher Tm similarly and make reporter closer to quencher resulting in better quenching effect, lower fluorescence background and higher signal-noise ratio. The probe is complete when it doesn't bind to the template, in addition, the fluorescence generating by reporter is absorbed by quencher, which leads to non-fluorescence. Otherwise, the probe is degraded by 5'→3' excision enzyme of Taq when it binds to the template, which leads to the separation between reporter and quencher, and then fluorescence is emerged. TaqMan-MGB probing method has some advantages such as higher sensitivity and specificity. The multiplex PCR in one tube, which can reduce the production cost and improve efficiency, can be realized by the alternative fluorescence group of different wavelength.

[Main components]

Every sample needs three reaction by using this kit, included 19M1(probe 19del 2235-2249, 2236-2250), 19M2(probe 19del 2240-2251, 2240-2257) and 21M(probe 2573T＞G、2582T＞A) detection liquids, the fluorescence signal is indicated by FAM and VIC. The components are shown in table one.

Number	Components	Size	Quantity
1	2×Taq Mix	1.2mL/test	1
2	ROX Reference Dye*1	48μL/ test	1
3	ROX Reference Dye Ⅱ*2	48μL/ test	1
4	19M1 reaction liquid	304μL/ test	1
5	19M2 reaction liquid	304μL/ test	1
6	21M reaction liquid	304μL/ test	1

7	Negative control	20μL/ test	6
8	Positive control	20μL/ test	2
9	product manual		1

Remarks: 1. Suitable for PRISM 7000/7700/7300 and StepOnePlus Real-Time PCR System; 2. Suitable for ABI PRISM 7500/7500 Fast Real-Time PCR System and Stratagene Mx3000P. Thermal Cycler Dice Real Time System, LightCycler and Smart Cycler Ⅱ System are not necessary to use ROX.

[Storage condition and validity]

2×Taq Mix and ROX Reference Dye (Ⅱ) should save at four degrees centigrade. The additional reagent should store at twenty degrees below zero. The validity of reagents in kit is about six months (Please use within the availability period).

[Self-prepared reagents]

Tips without RNase and DNase, PCR 96 pore plates or eight-linked reaction tubes.

[Suitable instrument]

ABI PRISM 7000/7700/7300, StepOnePlus Real-Time PCR System and so on.

[Requirement for samples]

1. DNA from serum, blood, plasma.
2. Tissue including fresh or embedded in paraffin, operation sample conserved in liquid nitrogen, ethyl alcohol and RNAlater.
3. Sample from puncture and biopsy.

DNA extraction: Using commercial kit to extract human genomic DNA such as QIAamp DNA Blood Mini Kit. Quantifying extracted DNA and adding less than100ng template in 20μL system.

[Test method]

We suggest that every sample should analyze together with the positive and negative control simultaneously.

1. Preparing reagent for reaction

Thawing nucleic acid reaction solution from the kit and centrifuging at 2000rpm for 10sec then.

2. Loading samples

a). Adding 7.6μL PCR reaction, 10μL 2×Taq Mix and 0.4μL ROX Reference Dye(Ⅱ) to every tube. Reaction liquid can be mixed well and packaged subsequently.

b). Adding 2μL DNA template, and quantifying DNA not more than 100ng firstly.

c). Centrifuging briefly all reagents in PCR 96 pore plates or eight reaction tubes.

3. PCR amplification

a). Putting PCR reaction plates on the fluorescence quantitative PCR instrument.

b). Setting the cycle working conditions

<ABI PRISM 7000/7700/7300 Real-Time PCR System：

95℃ 30sec；95℃ 5sec、60℃ 30～31sec（7700 30sec、7000/7300 31sec），40 cycles

<ABI StepOnePlus™ Real-Time PCR System：

95℃ 20sec；95℃ 1sec、60℃ 20sec，40 cycles

<ABI PRISM 7500/7500 Fast Real-Time PCR System：

95℃ 30sec；95℃ 3～5sec（7500 Fast 3sec、7500 5sec）、60℃ 30～34sec（7500 Fast 30sec、7500 34sec），40 cycles

< Thermsl Cycler Dice、LightCycler、Smart Cycler Ⅱ Real Time PCR System：

95℃ 30sec；95℃ 5sec、60℃ 20sec，40 cycles

c). Selection of the detection channels

Fluorescent groups are FAM and VIC. Fluorescent reference group is ROX. Fluorescent group of each our probe is indicated in table 2.

Detection of reaction liquid	Mutation type	Fluorescent group at the 5’ end of probe
19M1	19M1(1)	FAM
	19M1(2)	VIC
19M2	19M2	FAM
	19M3	VIC
21M	L858R	FAM
	L861Q	VIC

[Reference ranges]

Threshold is determined as the highest point of negative control.

[Explanation of detection results]

a).If the positive control and each test holes had no signal, the sample may be extracted unsuccessfully or little, or maybe exist PCR inhibitor. The sample should be extracted again until above results appeared.

b). Positive control has signal while test hole hasn’t, mutation is not existed.

c). Both the positive control and test hole have signals, mutation is existed.

[Limitation of detection method]

Probability analysis of false positive:

False positive may be resulted from reasons as follows: a). DNA degradation resulting from unreasonable sample collection, translocation and treatment or too little sample DNA from tumor cells. b).mutation in target sequence. c). some interference factor without validation or PCR inhibitor.

[Performance index of our kit]

1. The kit is complete and the capacity of reagents is right.

2. Specificity

The false positive will not appear when negative standard is detected.

3. Sensitivity

The false negative will not appear when positive standard is detected.

4. This kit can efficiently detect the EGFR mutation amount of about one ten-thousand in 200ng DNA .

[Attentions]

1. The detection result of this kit is only reference for medical theory research instead of clinical diagnosis and cure.

2. This kit should be transported and saved in low temperature. Before using it, please

make them fully dissolved and then centrifuge to mix well.

3. If the testing sample is tissue, please make sure the tissue have tumor cells. In addition, if the testing sample is fixed by formalin, the sample DNA may be loaded more for its degradation.

4. The experiment should be strictly set each operation region.

The first region: PCR preparation area----preparing reagents for PCR amplification.

The second region: detection and control sample treatment area

The third region: sample loading and detecting area----PCR amplification testing

All the things in every area are special use for avoiding pollution. Please clean the desk after experiment.

5. When packaging the reaction liquid, please avoid to generating bubbles. When loading samples, the samples should be dropped in reaction liquid totally but not adhered to the tube wall. After that, please cover the tube lid quickly. Besides, before PCR running, please also make sure all the tubes are fully closed against fluorescent substances pollution.

6. Experiment table and all laboratory supplies should be sterilized regularly by 1% sodium hypochlorite, 75%ethyl alcohol or ultraviolet lamp.

[Other matters]

Appendix I A list of essential goods in every zone

PCR preparation area	sample handling area	Sample testing area
1. Centrifuge	1. Centrifuge	1.Quantitative fluorescence instrument
2. Blending device	2. Blending device	2. ultraviolet lamp
3.Fridge（4℃、-20℃）	3.Fridge（4℃、-20℃）	
4. A set of micro sample injector	4. A set of micro sample injector	
5. centrifuge tube shelf	5. centrifuge tube shelf	
6. consumable supplies: disposable glove, tips, centrifugal tube	6. consumable supplies: disposable glove, tips, centrifugal tube	3. special coverall and Laboratory supplies
7. ultraviolet lamp, 75%ethyl alcohol	7. ultraviolet lamp, 75%ethyl alcohol	
8.special coverall and Laboratory supplies	8.special coverall and Laboratory supplies	

Appendix Ⅱ Six mutation types of EGFR in human

Mutation	Exon	Base Change	cosmic ID
L858R	21	2573T＞G	6224
L861Q	21	2582T＞A	6213
19M1(1)	19	2235-2249del15	6223
19M1(2)		2236-2250del15	6225

19M2	2240-2251del12	6210
19M3	2240-2257del18	12370

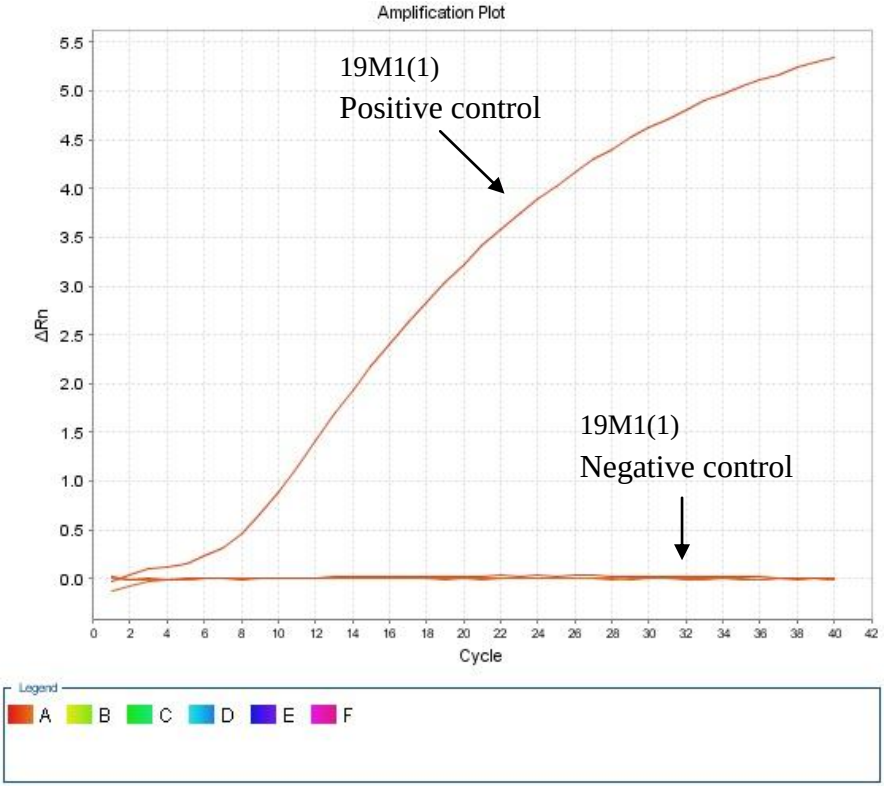

Figure 1 19M1(1) amplification curve of positive and negative control

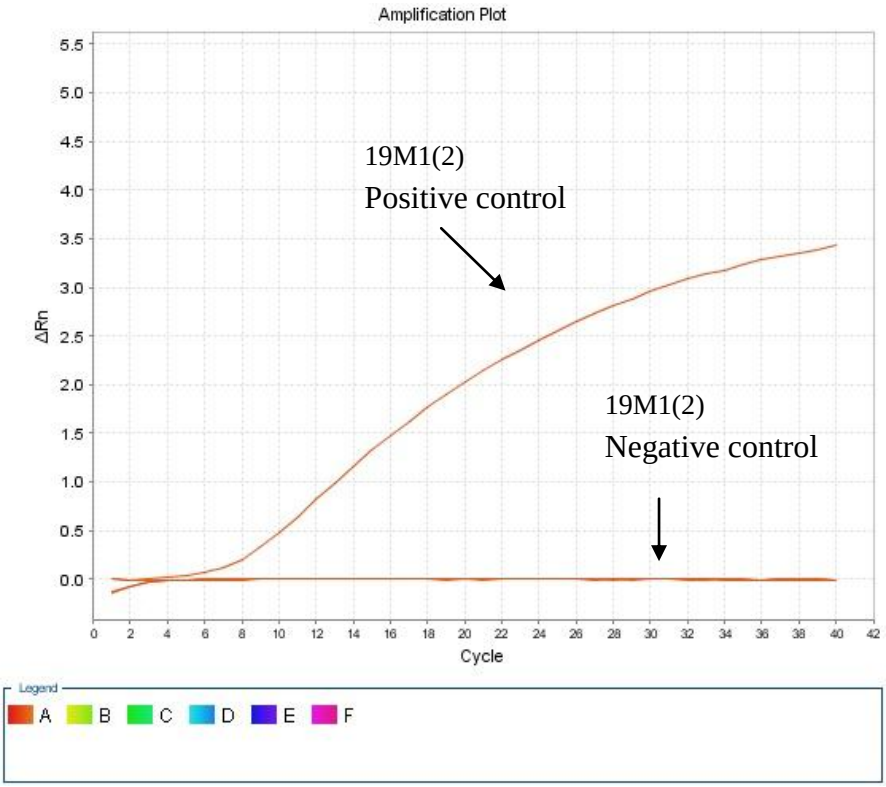

Figure 2 19M1(2) amplification curve of positive and negative control

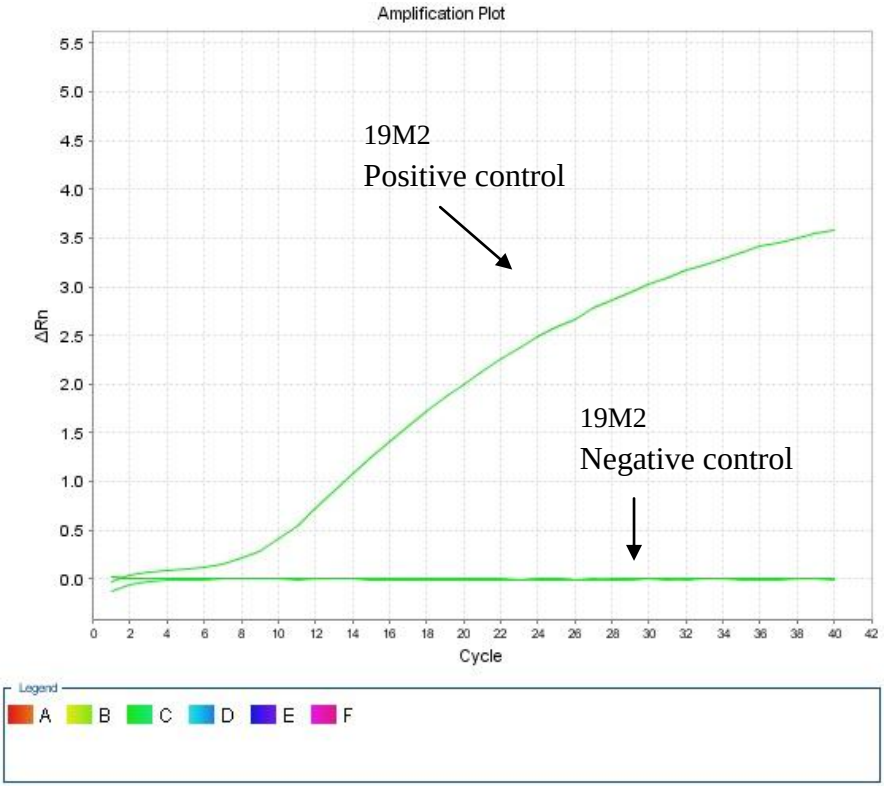

Figure 3. 19M2 amplification curve of positive and negative control

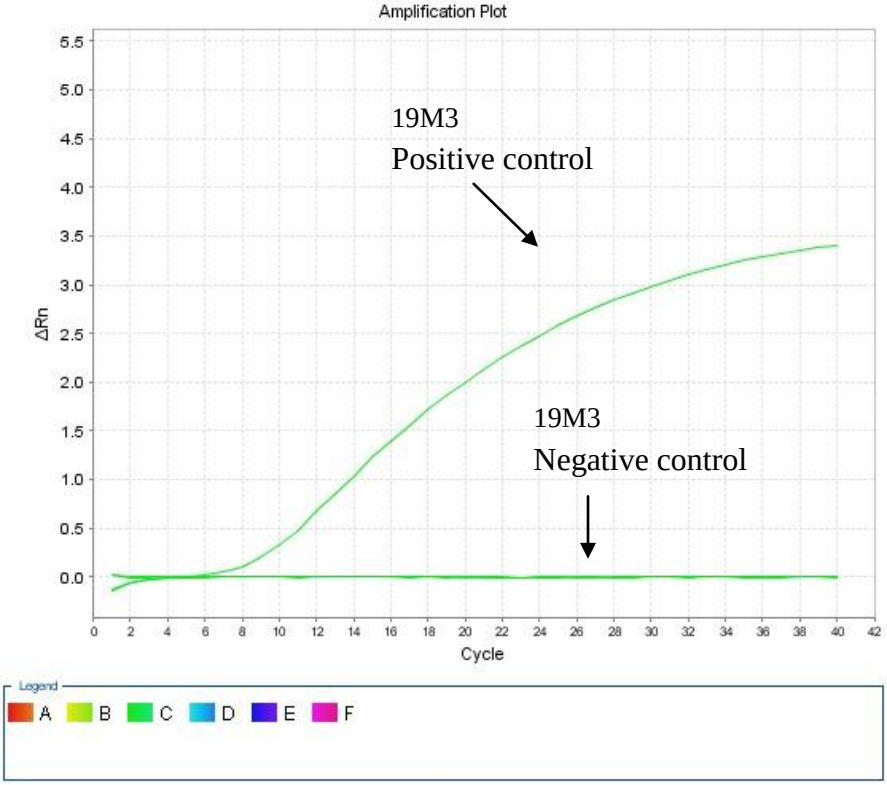

Figure 4. 19M3 amplification curve of positive and negative control

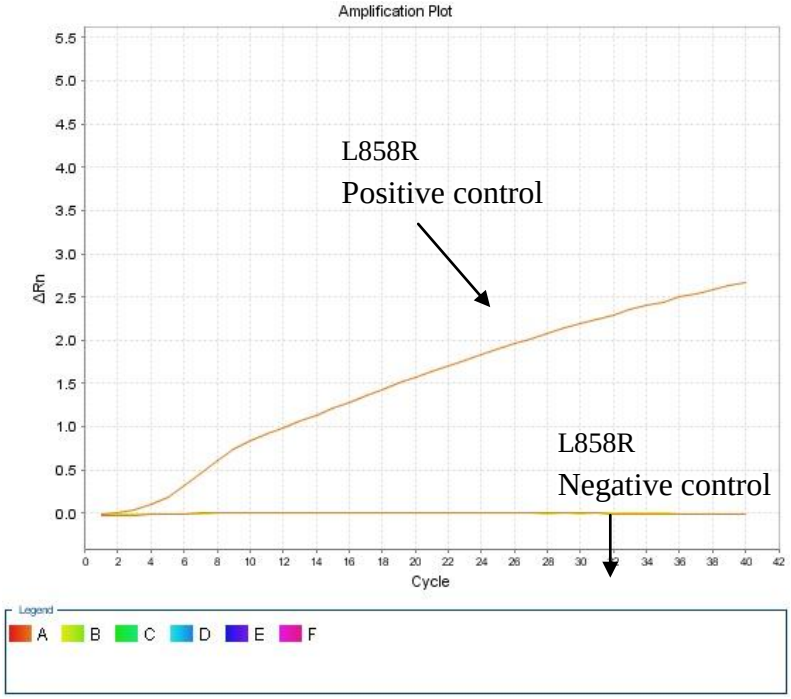

Figure 5. L858R amplification curve of positive and negative control

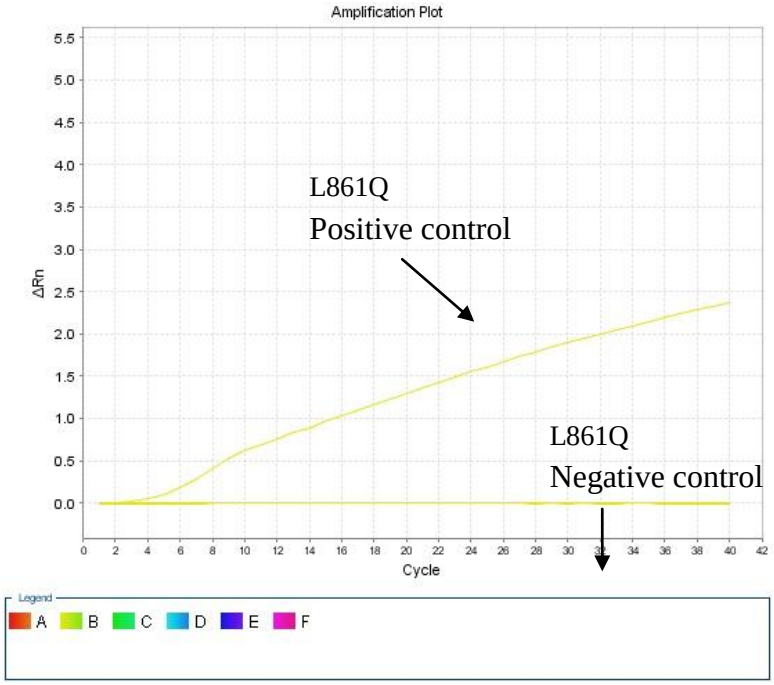

Figure 6. L861Q amplification curve of positive and negative control

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