AmoyDx
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AmoyDx® *EGFR* 29 Mutations Detection Kit

Instructions for Use

REF	8.01.0004	10 tests/kit	For Stratagene Mx3000P™, ABI7300, ABI7500, ABI7900HT
REF	8.01.0005	10 tests/kit	For LightCycler480, cobas® z480, Bio-Rad CFX96
REF	8.01.0006	10 tests/kit	For SLAN-96S

Amoy Diagnostics Co., Ltd.
No. 39, Dingshan Road, Haicang District,
361027 Xiamen, P. R. China
Tel: +86 592 6806835
Fax: +86 592 6806839
E-mail: sales@amoydx.com
Website: www.amoydx.com

EC REP **Qarad EC-REP BV**
Pas 257,
2440 Geel, Belgium

Version: P1.0
Mar 2022

Background

The epidermal growth factor receptor (EGFR) plays a central role in transmitting signals that promote cell growth and proliferation. Due to its association with malignancies, *EGFR* has become the target of an expanding class of anticancer therapies, such as gefitinib, erlotinib and afatinib, which are tyrosine kinase inhibitors (TKIs). The TKIs target the *EGFR* tyrosine kinase domain. These drugs work best on non-small cell lung cancer (NSCLC) patients whose cancer is driven by abnormal *EGFR* signaling. Lung cancer patients who experienced rapid, durable, complete or partial responses to TKI therapy have been found to harbor somatic mutations in *EGFR* gene. NSCLC patients with sensitizing *EGFR* mutations treated with TKI therapy have shown longer progression-free survival and higher response rate, compared with conventional chemotherapy. Resistance to TKI therapy, either in the primary tumor or acquired after TKI treatment, is associated with *EGFR* T790M mutation. Therefore, assessment of *EGFR* mutation status facilitates personalized treatment to lung cancer patients.

Intended Use

The AmoyDx® *EGFR* 29 Mutations Detection Kit is a real-time PCR assay for qualitative detection of 29 somatic mutations in exons 18, 19, 20 and 21 of *EGFR* gene in human genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue, or circulating DNA extracted from plasma/serum. The kit is intended to assess *EGFR* mutation status in NSCLC patients and aid in identifying patients who may respond to the treatment with an *EGFR*-TKI.

The kit is for *in vitro* diagnostic use, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit adopts Amplification Refractory Mutation System (ARMS) technology which comprises specific primers and fluorescent probes to detect gene mutations in real-time PCR assay. During the nucleic acid amplification, the targeted mutant DNA is matched with the bases at the 3' end of the primer, amplified selectively and efficiently, then the mutant amplicon is detected by fluorescent probes labeled with FAM. While the wild-type DNA cannot be matched with specific primers, there is no amplification occurring.

The kit is composed of *EGFR* Reaction Mix strips, *EGFR* Enzyme Mix and *EGFR* Positive Control.

1) The ***EGFR* Reaction Mix in Tubes** ①~⑦ includes mutation detection system and internal control systems. The mutation detection system includes primers and FAM-labeled probes specific for designated *EGFR* mutations, which is used to detect the *EGFR* mutation status. The internal control system contains primers and HEX-labeled probe for a region of genomic DNA without known mutations and polymorphism, to detect the presence of inhibitors and monitor the accuracy of the experimental operation.
2) The **External Control Reaction Mix in Tube** ⑧ of each **E*GFR* Reaction Mix** strip contains primers and FAM-labeled probe for a region of genomic DNA without known mutations and polymorphism, which is used to assess the quality of DNA.
3) The ***EGFR* Positive Control (PC)** contains a recombinant gene with *EGFR* mutations.
4) The ***EGFR* Enzyme Mix** contains Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.

Kit Contents

This kit contains the following materials:

Table 1 Kit Contents

Content	Main Ingredients	Quantity
***EGFR* Reaction Mix**	8-tube Strip*	12 strips
***EGFR* Enzyme Mix**	Taq DNA Polymerase, Uracil-N-Glycosylase	45 µL/tube ×1
***EGFR* Positive Control**	Plasmid DNA	250 µL/tube ×1

* Each strip (8-tube) includes the following contents for testing of one sample or one control (Table 2).

Table 2 Information of the 8-tube Strip

Tube No.	Reagent	Main Ingredients	Quantity	Fluorescent Signal
①	19-Del Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
②	L858R Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
③	T790M Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
④	Insertions Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
⑤	G719X Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
⑥	S768I Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
⑦	L861Q Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM, HEX/VIC
⑧	External Control Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	35 μL	FAM

Note:

Distinguish Tube ⑧ from Tube ① according to the hole position at the strip edge, described as follows.

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.

The shelf-life of the kit is twelve months. The maximal number of freeze-thaw cycles is five.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible Real-time PCR instrument:
   Stratagene Mx3000P™, ABI7300, ABI7500, ABI7900HT, LightCycler480, cobas® z480, Bio-Rad CFX96, or SLAN-96S.
2) DNA extraction kit. We recommend to use commercial extraction kits (e.g. Qiagen) for DNA extraction from FFPE tissue and plasma sample.
3) Spectrophotometer for measuring DNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Vortexer.
7) Nuclease-free centrifuge tubes.
8) Adjustable pipettors and filtered pipette tips for handling DNA.
9) Tube racks.
10) Disposable powder-free gloves.
11) Sterile, nuclease-free water.
12) 1×TE buffer (pH 8.0).

Precautions and Handling Requirements

For *in vitro* diagnostic use.

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.

- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid contact of the skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive results.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Use separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous DNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one-time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Setup the reaction volume as 40 μL.
- For Stratagene Mx3000P™, if there is a low net fluorescence signal (dR) but a high background signal (R), please reduce the signal gain setting of the instrument properly.
- For ABI instrument, please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
- For ABI7900HT, please set up as follows: Instrument: Standard, Ramp speed: Standard, Reaction volume: 40 μL. It's necessary to use the ABI7900 adaptor, available from BIOplastics, Cat No. 7900RAN.
- For LightCycler480 and cobas® z480 instrument, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.
- For SLAN-96S, please set up as follows: Probe mode: FAM, VIC. During the result analysis, open the "Preference" window, in "Chart Options" section; select "Selected Wells" for "Y-Axis Scaling Auto-adjust By" and "Absolute Fluorescence Value Normalization" for "Amplification Curve".
- Refer to the operations manual of the real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use, a fluorescence calibration should be conducted once a year.

Assay Procedure

1. DNA Extraction

The specimen material must be human genomic DNA extracted from FFPE tissue or plasma/serum samples. DNA extraction reagents are not included in the kit. Before DNA extraction, it is essential to use a standard pathology methodology to ensure tumor sample quality. Carry out the DNA extraction according to the instructions of the DNA extraction kit.

Tumor samples are not homogeneous, they may also contain non-tumor tissue. Data from different tissue sections of the same tumor may be inconsistent. DNA from non-tumor tissue may not contain detectable *EGFR* mutations. It's better to use tumor tissue samples with more than 30% tumor cells.

The OD_{260}/OD_{280} value of extracted DNA from FFPE tissue should be between 1.8 ~ 2.0 (measured using the spectrophotometer, the

NanoDrop 1000 /2000 spectrophotometer is recommended).

The amount of extracted DNA from FFPE tissue used for PCR amplification is shown in Table 3. And the circulating DNA isolated from plasma/serum should be used directly without dilution.

Table 3 Recommended DNA concentration

Tissue	Storage time	DNA concentration	DNA amount per reaction
FFPE tissue	≤ 3 months	1.5 ng/μL	7.05 ng
	> 3 months & ≤ 1 year	2 ng/μL	9.4 ng
	> 1 year & ≤ 3 years	2.5~3 ng/μL	11.75~14.1 ng

Note:

- *The FFPE tissue should be handled and stored properly. The storage time should preferably be less than 3 years.*
- *The plasma/serum samples should be derived from EDTA anti-coagulated peripheral whole blood.*
- *The extracted DNA should be used immediately. If not, it should be stored at -20±5℃ for no more than 3 months.*
- *Before detection, dilute the extracted tissue DNA with 1×TE buffer (pH 8.0) to designated concentration. We recommend using at least 5 μL DNA for 10 times dilution, to ensure the validity of final concentration.*

2. Mutation Detection

1) Take the ***EGFR* PC**, and ***EGFR* Enzyme Mix** out of the kit from the freezer, and other reagents remained in freezer at -20±5℃.
2) Thaw the ***EGFR* PC** at room temperature. When the reagent is completely thawed, mix each reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
3) Centrifuge ***EGFR* Enzyme Mix** for 5~10 seconds prior to use.
4) Take out the sample DNA (see Table 3 for DNA concentration) and nuclease-free water for NTC (No template control).
5) Prepare NTC mixture: pipet 42.3 μL nuclease-free water (NTC) and 2.7 μL *EGFR* Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.
6) Prepare sample DNA mixture: pipet 42.3 μL each sample DNA and 2.7 μL *EGFR* Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.
7) Prepare positive control (PC) mixture: pipet 42.3 μL *EGFR* Positive control and 2.7 μL *EGFR* Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.

   Note:

   - *Every PCR run must contain one Positive Control (PC) and one No Template Control (NTC).*
   - *The prepared mixtures should be used immediately, avoid prolonged storage.*
   - *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
   - *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*
8) Take out the 8-tube strips (***EGFR* Reaction Mix**) as needed (sufficient for samples, PC and NTC) and centrifuge the strips if there are any droplets in the caps of the PCR tubes. Then gently uncover the caps prior to use.
9) Add 5 μL of prepared NTC mixture to each PCR tube of NTC strip, and cap the PCR tubes.
10) Add 5 μL of each prepared sample DNA mixture to each PCR tube of sample strip, and cap the PCR tubes.
11) Add 5 μL of prepared PC mixture to each PCR tube of PC strip, and cap the PCR tubes.
12) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
13) Place the PCR tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 PCR Plate Layout

96 well layout												
Assay	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
19-Del	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
L858R	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
T790M	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC

Insertions	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
G719X	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
S768I	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
L861Q	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC
External Control	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Sample10	PC	NTC

14) Setup the PCR Protocol using the cycling parameters in Table 5.

Table 5 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	95℃	5min	/
2	15	95℃	25s	/
		64℃	20s	/
		72℃	20s	/
3	31	93℃	25s	/
		60℃	35s	FAM and HEX/VIC
		72℃	20s	/

15) Start the PCR run immediately.

16) When the PCR run is finished, analyze the data according to the “Results Interpretation” procedures.

3. Results Interpretation

Before analysis of mutation data, the following items should be checked:

1) For NTC: The FAM Ct values of Tubes ①~⑦ should be ≥31. If not, the data is *INVALID*. The sample should be retested.

2) For PC: The FAM Ct values of Tubes ①~⑧ and HEX/VIC Ct values of Tubes ①~⑦ should be < 20. If not, the data is *INVALID*. The sample should be retested.

3) For the internal control assay in Tubes ①~⑦ for each sample: The HEX/VIC Ct values should be < 31. If not, check the mutant FAM signals in Tubes ①~⑦ :
   a) If mutant FAM Ct value is < 31, continue with the analysis.
   b) If mutant FAM Ct value is ≥31, the data is *INVALID*. The sample should be retested.

4) For the external control assay in Tube ⑧ for each sample:
   a) The FAM Ct value should be between 13~21 for DNA extracted from FFPE tissues, and between 13~19 for DNA extracted from plasma/serum.
   b) If the FAM Ct value is <13, this indicates the DNA is overloaded. The DNA amount should be reduced and retested. But if the FAM Ct values of Tubes ①~⑦ are in Negative Ct range (see Table 6), the sample is determined as negative.
   c) If the FAM Ct value is >21 for DNA extracted from FFPE tissues or >19 for DNA extracted from plasma/serum, this indicates the DNA degradation or the presence of PCR inhibitors, or any error in experimental operation. The sample should be retested with increased or re-extracted DNA. But if any FAM Ct value of tubes ①~⑦ is < 26, the sample is determined as positive.

Analyze the mutation assay for each sample:

5) Record the FAM Ct values in Tubes ①~⑦ for each sample.

6) Check the mutant FAM Ct values in Tubes ①~⑦ according to Table 6:
   a) If any FAM Ct value of Tube ①~⑦ is < 26, the sample is determined as positive (*EGFR* mutation detected).
   b) If any FAM Ct value of Tubes ①~⑦ is in Acceptable Ct range, calculate the ΔCt value for each mutation showing positive amplification.
      i. **ΔCt value = Mutant FAM Ct value – External control FAM Ct value**.
      ii. If the ΔCt value is < the Cut-off ΔCt value, the sample is determined as positive (Mutation detected).

iii. If the ΔCt value is ≥ the Cut-off ΔCt value, the sample is determined as negative (No mutation detected) or under the LOD of the kit.

c) If all the FAM Ct values of Tubes ①~⑦ are in Negative Ct range or there is no amplification, the sample is determined as negative or under the LOD (Limit of Detection) of the kit.

Table 6 Result Determination

Mutation assay	19-Del	L858R	T790M	Insertions	G719X	S768I	L861Q	Results
Optimal Ct range	Ct<26	Ct<26	Ct<26	Ct<26	Ct<26	Ct<26	Ct<26	Positive.
Acceptable Ct range	26≤Ct<29	26≤Ct<29	26≤Ct<28	26≤Ct<29	26≤Ct<29	26≤Ct<29	26≤Ct<29	Interpret the results according to the ΔCt value.
Cut-off ΔCt value	12	11	7	9	7	8	8	
Negative Ct range	Ct≥29	Ct≥29	Ct≥28	Ct≥29	Ct≥29	Ct≥29	Ct≥29	Negative or under the LOD*.

* LOD: limit of detection

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7300, ABI7500, ABI7900HT, LightCycler480, cobas® z480, Bio-Rad CFX96, and SLAN-96S.

1) Analytical sensitivity:

Analytical sensitivity of the kit was established using plasmid DNA. 29 single *EGFR* mutant plasmid DNAs were diluted with 2 ng/μL wild-type DNA to prepare 29 plasmid DNAs of 1% mutant content. The 29 single *EGFR* mutant plasmid DNAs of 1% mutant content were tested for 20 repeats using three batches of AmoyDx® *EGFR* 29 Mutations Detection Kit, and come out a positive rate of at least 95%. Therefore, the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng sample DNA amount.

2) Specificity:

Specificity of the kit was established by testing 10 negative reference controls, which were prepared from 30 cases of NSCLC FFPE tissue samples with wild-type DNA confirmed by Sanger Sequencing. The test gave negative results and with 100% concordance rate.

3) Accuracy:

Accuracy of the kit was established by testing 29 *EGFR* positive reference controls, which were prepared from 29 cases of NSCLC FFPE tissue samples with *EGFR* mutations confirmed by Sanger Sequencing. The test gave corresponding positive results and with 100% concordance rate.

4) Precision:

3 precision controls: negative control, weak positive control (the mutant content is 5%) and strong positive control (the mutant content is 50%) were used in the validation. 3 batches of the kits were tested with the precision controls by 2 operators twice a day for 20 days on different PCR instruments. The Ct values were calculated, the CV values were all within 5%.

5) Interfering substance:

Two common interfering substances: hemoglobin and triglyceride, were evaluated in this study. It is confirmed that the potential maximum concentrations: 15 mg/mL hemoglobin and 37 mmol/L triglyceride would not interfere with the test result.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) The results can be used to assist clinical diagnosis, combined with other clinical and laboratory findings.
3) The kit has been validated for use with DNA extracted from FFPE tissue and plasma/serum samples.
4) The kit can only detect the 29 *EGFR* mutations listed in the appendix.
5) Reliable results are dependent on proper sample processing, transport, and storage.
6) The sample containing degraded DNA may affect the ability of the test to detect *EGFR* mutation.
7) Samples with negative result (No mutation detected) may harbor EGFR mutations not detected by this assay.
8) Circulating DNA extracted from plasma or serum with negative results (No Mutation detected) may harbor *EGFR* mutation, which could be confirmed with matched tissue DNA detection.

References

1) Han JY, Park K, Kim SW, *et al*. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung [J]. *J Clin Oncol*, 2012, 30 (10):1122-1128.

2) Zhou C, Wu YL, Chen G, *et al*. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation- positive non-small-cell lung cancer (OPTIMAL,CTONG-0802): a multicentre, open-label, randomised, phase 3 study[J].*Lancet Oncol*, 2011,12 (8):735-742.

3) Gazdar A F, Shigematsu H, Herz J, *et al*. Mutations and addiction to EGFR: the Achilles 'heal'of lung cancers? [J]. *Trends in molecular medicine*, 2004, 10(10): 481-486.

4) Lynch T J, Bell D W, Sordella R, *et al*. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib[J]. *New England Journal of Medicine*, 2004, 350(21): 2129-2139.

5) Paez J G, Jänne P A, Lee J C, *et al*. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy[J]. *Science*, 2004, 304(5676): 1497-1500.

6) Pao W, Miller V, Zakowski M, *et al*. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib[J]. *Proceedings of the National Academy of Sciences of the United States of America*, 2004, 101(36): 13306-13311.

7) Helena A Y, Arcila M E, Rekhtman N, *et al*. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers[J]. *Clinical cancer research*, 2013, 19(8): 2240-2247.

8) Huang Z, Wang ZJ, Bai H, *et al*; The detection of EGFR mutation status in plasma is reproducible and can dynamically predict the efficacy of EGFR-TKI. *Thoracic Cancer*, 2012, 3(4): 334-340.

Symbols

EC REP	Authorized Representative in the European Community	IVD	In Vitro Diagnostic Medical Device
	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use By
Σ	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		

Appendix

***EGFR* Mutations Detected by the Kit**

Tube No.	Reagent	Exon	Mutation	Base Change	Cosmic ID
①	19-Del Reaction Mix	19	E746_A750del (1)	2235_2249del15	6223
			E746_A750del (2)	2236_2250del15	6225
			L747_P753>S	2240_2257del18	12370
			E746_T751>I	2235_2252>AAT(complex)	13551
			E746_T751del	2236_2253del18	12728
			E746_T751>A	2237_2251del15	12678
			E746_S752>A	2237_2254del18	12367
			E746_S752>V	2237_2255>T(complex)	12384
			E746_S752>D	2238_2255del18	6220
			L747_A750>P	2238_2248>GC(complex)	12422
			L747_T751>Q	2238_2252>GCA(complex)	12419
			L747_E749del	2239_2247del9	6218
			L747_T751del	2239_2253del15	6254
			L747_S752del	2239_2256del18	6255
			L747_A750>P	2239_2248TTAAGAGAAG>C(complex)	12382
			L747_P753>Q	2239_2258>CA(complex)	12387
			L747_T751>S	2240_2251del12	6210
			L747_T751del	2240_2254del15	12369
			L747_T751>P	2239_2251>C(complex)	12383
②	L858R Reaction Mix	21	L858R	2573T>G	6224
③	T790M Reaction Mix	20	T790M	2369C>T	6240
④	Insertions Reaction Mix	20	H773_V774insH	2319_2320insCAC	12377
			D770_N771insG	2310_2311insGGT	12378
			V769_D770insASV	2307_2308insGCCAGCGTG	12376
⑤	G719X Reaction Mix	18	G719A	2156G>C	6239
			G719S	2155G>A	6252
			G719C	2155G>T	6253
⑥	S768I Reaction Mix	20	S768I	2303G>T	6241
⑦	L861Q Reaction Mix	21	L861Q	2582T>A	6213