AmoyDx® *EGFR/ALK/ROS1* Mutations Detection Kit

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

REF	8.01.0079	8 tests/kit	For Stratagene Mx3000P™
REF	8.01.0080	8 tests/kit	For LightCycler480 II
REF	8.01.0081	8 tests/kit	For SLAN-96S
REF	8.01.0082	8 tests/kit	For ABI7500

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Background

Lung cancer is the most common malignant tumor and the leading cause of cancer death worldwide. About 80~85% of lung cancers are non-small cell lung cancer (NSCLC). In recent years, various molecular targeted therapies have been developed for the treatment of advanced lung cancer. Targeted therapies are potentially very effective in patients with specific gene mutations or rearrangements. The identification of multiple genetic abnormalities which drive oncogenic signaling pathways within cancer cells has led to the development of new targeted therapies in a subset of patients with NSCLC. Like *EGFR* mutations, *ALK* and *ROS1* gene fusions are shown to be effective therapeutic targets of tyrosine kinase inhibitors (TKIs).

It has been reported that lung cancer patients who experienced rapid, durable, complete or partial responses to EGFR-TKIs therapy have been found to harbor somatic mutations in the *EGFR* gene, and the presence of the *ALK* and *ROS1* gene fusions are correlated with the efficacy of TKI therapy (e.g. crizotinib). Based on analysis of tumor DNA and RNA, *EGFR* mutations, *ALK* gene fusions and *ROS1* gene fusions can be detected by real-time PCR method.

Intended Use

The AmoyDx® *EGFR/ALK/ROS1* Mutations Detection Kit is an *in vitro* nucleic acid amplification test intended for qualitative detection of 24 *EGFR* mutations (exons 18-21), 21 *ALK* gene fusions and 13 *ROS1* gene fusions in tumor DNA/RNA extracted from NSCLC tissue samples.

The kit is for research use only, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit contains RNA gene fusion and DNA gene mutation detection systems.

The gene fusion detection system is based on two major processes: 1) **Reverse Transcription**: extracted RNA from FFPE tumor tissue is employed in this step, reverse transcription of target RNA enables complementary DNA (cDNA) synthesis with the action of reverse transcriptase and specific primers. 2) **PCR Amplification**: the specific primers are designed for amplification of cDNA of *ALK* and *ROS1* fusions, and the fusion amplicon is detected by fluorescent probes.

The gene mutation detection system adopts ADx-ARMS technology, which comprises specific primers and fluorescent probes to detect mutations and reference gene in a real-time PCR assay. The mutant DNA is amplified accurately by the specific primers, and detected by the fluorescent probes.

The kit is composed of EAR Reaction Mix strips, EAR Positive Control, EAR Enzyme Mix A, EAR Enzyme Mix B.

1) The **EAR Reaction Mix 1~3** includes an RNA detection system and an internal control system for RNA samples. The RNA detection system contains primers and FAM-labeled probes specific for *ALK* gene fusions (**Reaction Mix 1**) and *ROS1* gene fusions (**Reaction Mix 2-3**). The internal control system contains primers and HEX (VIC)-labeled probe for detection of reference gene *HPRT1*, to detect the presence of inhibitors and monitor the accuracy of experimental operation, which may lead to false negative results.
2) The **EAR Reaction Mix 4~7** includes mutant DNA detection system and an internal control system for DNA samples. The mutant DNA detection system contains primers and FAM-labeled probes specific for *EGFR* gene mutations. The internal control system contains primers and HEX (VIC)-labeled probe for detection of a region of genomic DNA that has no known mutations or SNPs, to detect the presence of inhibitors and monitor the accuracy of experimental operation, which may lead to false negative results.
3) The **EAR Reaction Mix 8** is used as an external control, which contains primers and FAM-labeled probes for detection of a region of genomic DNA that has no known mutations or SNPs, to assess the quality of DNA.
4) The **EAR Positive Control (PC)** contains a recombinant gene with *EGFR* mutations, *ALK* gene fusions, and *ROS1* gene fusions.
5) The **EAR Enzyme Mix A** contains the reverse transcriptase for reverse transcription of target RNA and reference gene RNA into cDNA, the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase to prevent PCR amplicon carryover contamination.
6) The **EAR Enzyme Mix B** contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase to prevent PCR amplicon carryover contamination.

Kit Contents

The kit contains the following materials (Table 1).

Table 1 Kit Contents

Content	Main Ingredients	Quantity
EAR Reaction Mix	8-tube strip*	10 strips
EAR Enzyme Mix A	Reverse Transcriptase Taq DNA Polymerase, Uracil-N-Glycosylase	45 µL/tube ×1
EAR Enzyme Mix B	Taq DNA Polymerase, Uracil-N-Glycosylase	30 µL/tube ×1
EAR Positive Control–	Plasmid DNA	300 µL/tube ×1

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

Table 2 Information of the 8-tube strip

Tube No.	Reagent	Detected Target	Main Ingredients	Quantity	Fluorescent Signal
①	**EAR Reaction Mix 1**	*ALK* Fusion	Primers, Probes, Mg^{2+}, dNTPs	30 µL	FAM, HEX/VIC
②	**EAR Reaction Mix 2**	*ROS1* Fusion	Primers, Probes, Mg^{2+}, dNTPs	30 µL	FAM, HEX/VIC
③	**EAR Reaction Mix 3**	*ROS1* Fusion	Primers, Probes, Mg^{2+}, dNTPs	30 µL	FAM, HEX/VIC
④	**EAR Reaction Mix 4**	*EGFR* Mutation	Primers, Probes, Mg^{2+}, dNTPs	35 µL	FAM, HEX/VIC
⑤	**EAR Reaction Mix 5**	*EGFR* Mutation	Primers, Probes, Mg^{2+}, dNTPs	35 µL	FAM, HEX/VIC
⑥	**EAR Reaction Mix 6**	*EGFR* Mutation	Primers, Probes, Mg^{2+}, dNTPs	35 µL	FAM, HEX/VIC
⑦	**EAR Reaction Mix 7**	*EGFR* Mutation	Primers, Probes, Mg^{2+}, dNTPs	35 µL	FAM, HEX/VIC
⑧	**EAR Reaction Mix 8**	External Control	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 eight months. The maximal number of freeze-thaw cycle is five.

Materials Required But Not Supplied

1) Compatible PCR instruments:
   Stratagene Mx3000P™, ABI7500, LightCycler480 II, or SLAN-96S.
2) DNA/RNA extraction kit. We recommend to use AmoyDx® FFPE DNA/RNA Kit for FFPE tissue specimens.
3) Spectrophotometer for measuring DNA/RNA 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/RNA.
9) Tube racks.
10) Disposable powder-free gloves.
11) Sterile, nuclease-free water.
12) 1×TE buffer (pH 8.0).

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. 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 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 nucleic acid 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™, please set up the Fliter Set Gain Settings of FAM and HEX-JOE as 2.
- For ABI instrument, please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
- For LightCycler480 II, if there is fluorescence crossover on the instrument, fluorescence calibration is also required. To run the assays on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.
- For SLAN-96S instrument, please set up as follows: Fluorophores/Dyes: FAM, VIC. During the result interpretation, select “Selected Wells” for “Y-Axis Scaling Auto-adjust By” and “Absolute fluorescence Method” for “Normalization algorithm”.
- Refer to the operation manual of the real-time PCR instrument for detailed instructions.
- We recommend that all PCR instruments in use, a fluorescence calibration should be conducted once a year.

Assay Procedure

1. DNA/RNA Extraction

The specimen material must be human genomic DNA and total RNA extracted from formalin-fixed paraffin-embedded (FFPE) samples. DNA/RNA extraction kit is not included in the kit. Carry out the DNA and RNA extraction according to the instructions of DNA/RNA extraction kit.

Before extraction, it is essential to use a standard pathology methodology to ensure tumor sample quality. Tumor samples are

non-homogeneous, may also contain non-tumor tissue. Data from different tissue sections of the same tumor may be inconsistent. RNA/DNA from non-tumor tissue would not be detected with *ALK* /*ROS1* fusions or *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 should be between 1.8~2.1 (measured using the spectrophotometer, the NanoDrop 1000 /2000 spectrophotometer is recommended).

The Total RNA concentration should be between 10 ~500 ng/μL.

The amount of extracted DNA from FFPE tissue used for EGFR mutation detection differs according to different storage time (see Table 3).

Table 3 Recommended DNA concentration

Tissue	Storage time	DNA concentration	DNA amount per reaction
FFPE	≤ 3 months	1.5 ng/μL	7.5 ng
	> 3 months & ≤ 1 year	2 ng/μL	10 ng
	> 1 year & ≤ 2 years	2.5~3 ng/μL	12.5~15 ng

Note:

- *The FFPE tissue should be handled and stored properly. The storage time should preferably be less than 2 years.*
- *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.*
- *The extracted DNA should be used immediately. If not, it should be stored at -20±5℃ for no more than 6 months.*
- *The extracted RNA should be used immediately. If not, it should be stored at -20±5℃ for no more than one week.*

2. Mutation/Fusion Detection

1) Take the **EAR Positive Control (PC)**, **EAR Enzyme Mix A** and **EAR Enzyme Mix B** out of the kit from the freezer to the room temperature.
2) When the **EAR PC** is completely thawed, mix the reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
3) Centrifuge **EAR Enzyme Mix A** and **EAR Enzyme Mix B** for 5~10 seconds prior to use.
4) Preparation of **Sample-Mix A** and **Sample-Mix B**: for each sample, add 3.5 μL **EAR Enzyme Mix A** into 31.5 μL sample RNA to obtain **Sample-Mix A**, and add 1.8 μL **EAR Enzyme Mix B** into 28.2 μL sample DNA (refer to Table 3 for DNA concentration) to obtain **Sample-Mix B**. Thoroughly mix each Mix by vortexing and centrifuge for 5~10 seconds.
5) Preparation of **PC-Mix A** and **PC-Mix B**: add 3.5 μL **EAR Enzyme Mix A** into 31.5 μL EAR positive control to obtain **PC-Mix A**, and add 1.8 μL **EAR Enzyme Mix B** into 28.2 μL EAR positive control to obtain **PC-Mix B**. Thoroughly mix each Mix by vortexing and centrifuge for 5~10 seconds.
6) Preparation of **NTC-Mix A** and **NTC-Mix B**: add 3.5 μL **EAR Enzyme Mix A** into 31.5 μL nuclease-free water (No Template Control, NTC) to obtain **NTC-Mix** A, and add 1.8 μL **EAR Enzyme Mix B** into 28.2 μL nuclease-free water to obtain **NTC-Mix B**. Thoroughly mix each Mix by vortexing and centrifuge for 5~10 seconds.

   Note:

   - *Every PCR run must contain one PC and one 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.*
7) Take out the **EAR Reaction Mix** strips and centrifuge the strips if there are any reagent droplets in the lid of the PCR tubes. Then briefly uncover the caps prior to use.
8) For NTC strip, transfer 10 μL of **NTC-Mix A** to tubes ①~③ respectively, transfer 5 μL of **NTC-Mix B** to tubes ④~⑧ respectively. Cap the PCR tubes.
9) For Sample strip, transfer 10 μL of **Sample-Mix A** to tubes ①~③ respectively, transfer 5 μL of **Sample-Mix B** to tubes ④~⑧ respectively. Cap the PCR tubes.
10) For PC strip, transfer 10 μL of **PC-Mix A** to tubes ①~③ respectively, transfer 5 μL of **PC-Mix B** to tubes ④~⑧ respectively. Cap

the PCR tubes.

11) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.

12) Place the PCR tubes into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 Recommended PCR Plate Layout

Well	1	2	3	4	5	6	7	8	9	10
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
C	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
E	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
F	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
G	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC
H	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	PC	NTC

13) Setup the PCR protocol using the cycling parameters in Table 5.

Table 5 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	42℃	5 min	/
		95℃	5 min	/
2	10	95℃	25 s	/
		64℃	20 s	/
		72℃	20 s	/
3	36	93℃	25 s	/
		60℃	35 s	FAM and HEX(VIC)
		72℃	20 s	/

14) Start the PCR run immediately.

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

3. Results Interpretation

Before sample data analysis, the following items should be checked:

1) For NTC: The FAM Ct values in Tubes ①~⑦ should be ≥36. If not, the data is *INVALID*. The sample should be retested.

2) For PC: The FAM Ct value in Tube ①~⑧ and HEX/VIC Ct value in Tube ①~⑦ should be < 30. If not, the data is *INVALID*. The sample should be retested.

Analyze the fusion/mutation assay for each sample:

3) Analysis of *ALK* and *ROS1* gene fusion assay results in Reaction Mix 1~3:

   a) Check the HEX/VIC signals in Tube ①~③ for each sample:

   - If all the HEX/VIC Ct values in Tube ①~③ ≤27, then continue with further analysis.
   - If any of HEX/VIC Ct value in Tube ①~③ >27, this indicates any error in experimental operation, or the presence of PCR inhibitors, or sample RNA degradation. But if there is positive FAM Ct value, the result is believable; otherwise, we suggest re-extracting the RNA and doing this experiment again.

   b) Check the FAM signals in Tube ①~③ for each sample:

   - If all the FAM Ct value in Tube ①~③ ≥35, the sample is determined as Negative (No *ALK&ROS1* fusion detected) or under the LOD (limit of Detection) of the kit.
   - If the FAM Ct value in Tube ① <35, the sample is determined as *ALK* positive.
   - If any FAM Ct value in Tube ②~③ <35, the sample is determined as *ROS1* positive.

4) Analysis of *EGFR* mutation assay results in Reaction Mix 4~8:

   a) Check the HEX/VIC signals in Tube ④~⑦ for each sample, the HEX/VIC Ct should be ≤36.

b) Check the FAM signal of the external control in Tube ⑧ for each sample:

- The FAM Ct value in Tube ⑧ should be between 20~26.
- If the FAM Ct value in Tube ⑧ <20, this indicates the DNA is overloaded. The DNA amount should be reduced and retested. But if FAM Ct values of Tubes ④~⑦ are in Negative Ct range (see Table 6), the sample is determined as negative.
- If the FAM Ct value in Tube ⑧ >26, 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 FAM Ct values of Tubes ④~⑦ is <31, the sample is determined as positive.

c) Check the FAM signals in Tube ④~⑦ for each sample. Determine the result according to Table 6.

- If the mutant FAM Ct value in Tube ④~⑦ is <31, the sample is determined as positive (*EGFR* mutation detected).
- If the mutant FAM Ct value in Tube ④~⑦ falls in Acceptable Ct range, calculate the ΔCt value:
  - If the ΔCt value is < the ΔCt Cut-off value, the sample is determined as positive.
  - If the ΔCt value is ≥ the ΔCt Cut-off value, the sample is determined as negative or below the limits of the kit.
  - The calculation of ΔCt: **ΔCt = mutant FAM Ct value – external control FAM Ct value**.
- If the mutant FAM Ct values in Tubes ④~⑦ are in Negative Ct range or there is no amplification, the sample is determined as negative or below the detection limit of the kit.

Table 6 Results Determination

Tube No.	④	⑤	⑥	⑦	**Results**
Mutation Name	19-Del	L858R	T790M	G719A, G719C, L861Q	
Optimal Ct range	Ct<31	Ct<31	Ct<31	Ct<31	Positive
Acceptable Ct range	31≤Ct<34	31≤Ct<34	31≤Ct<33	31≤Ct<33	Interpret the results according to the ΔCt value
Cut-off ΔCt value	11	11	7	9	
Negative Ct range	Ct≥34	Ct≥34	Ct≥33	Ct≥33	Negative or under LOD*

* LOD: limit of detection

5) The sample may contain two or more mutations or fusion patterns simultaneously.

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7500, LightCycler480 II, and SLAN-96S.

1) Analytical Sensitivity:
   a) For sample DNA, the kit allows detection of 1% *EGFR* mutant DNA 1% mutant DNA in a background of 99% normal DNA at 7.5~15 ng sample DNA amount.
   b) For sample RNA, the kit allows detection of 450 copies armored RNA including *ALK* and *ROS1*gene fusions at 0.09~4.5 μg sample RNA amount.
2) Specificity:
   The specificity of the kit was established by testing negative reference controls. The test gave negative results and negative concordance rate was 100%.
3) Accuracy:
   Accuracy of the kit was established by testing positive reference controls. The test gave positive results and positive concordance rate was 100%.
4) Precision:
   Precision of the kit was established by performing a certain mutant positive reference control for 10 repeats; CV (coefficient of variation) of ≤10%.
5) Interfering substances:
   Two common potential interfering substances hemoglobin and triglyceride were evaluated. It is confirmed that the potential maximum concentrations: 2 g/L 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 kit has been validated for use with extracted DNA and RNA from NSCLC patient FFPE tissue.
3) The kit can only detect 24 *EGFR* mutations, 21 *ALK* gene fusions and 13 *ROS1* gene fusions listed in the appendix.
4) Reliable results are dependent on proper sample processing, transport, and storage.
5) The sample containing degraded DNA/RNA may affect the ability of the test to detect gene mutations or fusions.
6) Samples with negative result may harbor *EGFR* mutation or *ALK/ROS1* fusions not detected by this assay.

References

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Symbols

	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use-by Date
Σ	Contains Sufficient for <n> Tests		Temperature Limit
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		

Appendix

***EGFR* Mutations, *ALK* and *ROS1* Gene Fusions Detected by the Kit**

Tube No.	Detect target	Exon	Mutation or Fusion	Mutation or Fusion		Cosmic ID
①	*ALK* Fusion	/	*EML4-ALK* *KIF5B-ALK* *KLCL1-ALK* *TFG-ALK*	E2;A20	E2;ins117A20	/
				E3;ins53A20	E6;A19	
				E6;A20	E6ins33;A20	
				E6;ins18A20	E13;A20	
				E13;ins69A20	E17;ins30A20	
				E17ins61;ins34A20	E17ins65;A20	
				E17;ins68A20	E17del58;ins39A20	
				E18;A20	E20;A20	
				E20;ins18A20		
				KI17;A20	KI24;A20	
				KL9;A20	T4;A20	
②	*ROS1* Fusion	/	*SLC34A2-ROS1* *CD74-ROS1* *SDC4-ROS1* *EZR-ROS1*	SL4;R32	SL14del;R32	/
				SL4;R34	SL14del;R34	
				CD6;R32	CD6;R34	
				SD2;R32	SD4;R32	
				SD4;R34	EZ10;R34	
③	*ROS1* Fusion	/	*TPM3-ROS1*	TP8;R35		/
			LRIG3-ROS1	L16;R35		
			GOPC-ROS1	GO8;R35		
④	*EGFR* Mutation (19-Del)	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
⑤	*EGFR* Mutation	21	L858R	2573T>G		6224
⑥	*EGFR* Mutation	20	T790M	2369C>T		6240
⑦	*EGFR* Mutation	18	G719A	2156G>C		6239
		18	G719C	2155G>T		6253
		21	L861Q	2582T>A		6213