# AmoyDx® Pan Lung Cancer PCR Panel

**Detection of 167 hotspot variants in *EGFR/ALK/ROS1/KRAS/BRAF/HER2/RET/MET/NTRK1/NTRK2/NTRK3* genes**

Instruction for Use

REF 8.0131201W008J 8 tests For QuantStudio 5, QuantStudio Dx

**Amoy Diagnostics Co., Ltd.**
39 Dingshan Road, Haicang District,
Xiamen 361027, P. R. China
Tel: +86 592 6806835
Fax: +86 592 6806839
E-mail: sales@amoydx.com
Website: http://www.amoydx.com

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

Version: P1.5
January 2021

## Background

Lung cancer is one of the most common malignant tumors, and 80~85% of lung cancers are non-small cell lung cancer (NSCLC). There are many driver mutations in NSCLC. The frequency of mutations in patients with NSCLC for the *EGFR* gene is 10~50% [1], for *HER2* 1~4% [2-3], for *KRAS* 5~25% [4-6], and for *BRAF* 1~2% [7-8]. About 3~7% [9-12] of NSCLC patients have gene fusions in *ALK* 1% [13-14] in ROS1, 1% [13, 15-17] in RET, 0.12% [18] in NRTK1, 0.02% [18] in NTRK2, and 0.08% [18] in *NTRK3* genes. Approximately 1% of lung adenocarcinoma patients harbor *MET* exon 14 skipping mutations [19]. Targeted therapies have been developed and approved for use in patients whose tumors have some of the genomic alterations seen in NSCLC. For instance, there are approved *EGFR* inhibitors [20-21], *ALK* inhibitors [22-23], *ROS1* inhibitors [24-25], *NTRK* inhibitors [25-27] and *BRAF* inhibitors [7, 28] for patients with specific genomic alterations in these genes. According to different treatment guidelines in the world including the NCCN guidelines [29] testing for genomic alterations is a requirement in order to identify patients that may benefit from these targeted therapies. Furthermore, there are many drugs in late stage development or recently approved for other alterations such as *RET* [30], *MET* [31], *HER2* [32], and *KRAS* [33].

## Intended Use

The AmoyDx® Pan Lung Cancer PCR Panel is a real-time PCR assay for qualitative detection of 167 hotspot alterations in *EGFR*, *ALK*, *ROS1*, *KRAS*, *BRAF*, *HER2*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* genes. The kit is intended to be used to aid clinician to identify the multi-gene status of NSCLC patients.

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

## Principles of the Procedure

This kit contains a RNA gene fusion detection system in LEG Reaction Mix A and a DNA gene mutation detection system in LEG Reaction Mix B.

The RNA gene fusion detection includes two processes:

1) Reverse Transcription: extracted RNA from FFPE or fresh 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, and *ALK*, *ROS1*, *RET*, *MET*, *NTRK1*, *NTRK2* and *NTRK3* variant amplicon is detected by fluorescent probes.

The DNA gene mutation detection system uses ADx-ARMS technology, which comprises specific primers and fluorescent probes to detect gene mutations. During the amplification, the target mutant DNA is matched with the bases at the 3' end of the primer, and amplified efficiently, then the mutant amplicon is detected by fluorescent-labeled probes. While the wild-type DNA cannot be matched with specific primers, there is no amplification occurring.

The kit contains LEG Reaction Mix A strips, LEG Reaction Mix B strips, LEG RT Reaction Mix, sufficient positive control and enzyme.

1) **LEG Reaction Mix A strips** are designed for RNA fusion detection and internal control detection. The LEG Reaction Mixes A1~A8 include primers and FAM-labeled probes specific for detection of *ALK, NTRK1, NTRK2, NTRK3, ROS1, RET* gene fusions and *MET* exon14 skipping mutations. The LEG Reaction Mixes A4/A8 also contain primers and VIC-labeled probes for detection of housekeeping gene *HPRT1* as reference gene to assess the RNA quality.
2) **LEG Reaction Mix B strips** are designed for DNA mutation detection and external control detection. The Reaction Mixes B1~B7 include primers and FAM/VIC/ROX-labeled probes specific for detection of hotspot mutations in *EGFR*, *HER2*, *KRAS,* and *BRAF* genes. The LEG Reaction Mix B8 contains an external control reaction mix, which is composed of primer and FAM/VIC/ROX-labeled probes for detection of a region of genomic DNA that has no known mutations or polymorphisms to assess the DNA quality.
3) The **LEG RT Reaction Mix I** contains primers specific for reverse transcription of mRNA of *ALK, NTRK1, NTRK2, NTRK3* gene, and reference genes into cDNA.
4) The **LEG RT Reaction Mix II** contains primers specific for reverse transcription of mRNA of *ROS1*, *RET*, *MET* gene, and reference genes into cDNA.
5) The **LEG Reverse Transcriptase** is for reverse transcription of mRNA of target genes and reference genes into cDNA.
6) The **LEG Enzyme Mix A** and **LEG Enzyme Mix B** contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase, which works at room temperature to prevent PCR amplicon carryover contamination.
7) The **LEG Positive Control** contains recombinant gene with *EGFR*, *KRAS*, *BRAF*, *HER2*, *ALK*, *ROS1*, *RET*, *MET*, *NTRK1*, *NTRK2,*

and *NTRK3* alternations.

## Kit Contents

This kit contains the following materials:

Table 1 Kit Contents

| Content | Main Ingredients | Quantity |
|---|---|---|
| **LEG Reaction Mix A (LEG FU)** | Primers, probe, $Mg^{2+}$, dNTPs | 8-tube strip* ×12 |
| **LEG Reaction Mix B (LEG MU)** | Primers, probe, $Mg^{2+}$, dNTPs | 8-tube strip* ×12 |
| **LEG RT Reaction Mix I** | Primers, $Mg^{2+}$, dNTPs | 220 μL/tube ×1 |
| **LEG RT Reaction Mix II** | Primers, $Mg^{2+}$, dNTPs | 220 μL/tube ×1 |
| **LEG Reverse Transcriptase** | Reverse Transcriptase | 16 μL/tube ×1 |
| **LEG Enzyme Mix A** | Taq DNA Polymerase, Uracil-N-Glycosylase | 45 μL/tube ×1 |
| **LEG Enzyme Mix B** | Taq DNA Polymerase, Uracil-N-Glycosylase | 45 μL/tube ×1 |
| **LEG Positive Control** | Plasmid DNA | 500 μL/tube ×1 |

*Each strip (8-tube) includes the following contents (Table 2):

Table 2 Information of **LEG Reaction Mix A**

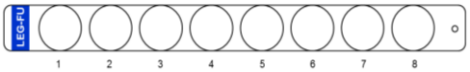
| Tube No. | Reagent | Target to detect | Quantity | Florescence Signal |
|---|---|---|---|---|
| ① | LEG Reaction Mix A1 | *ALK* Fusions | 35 μL | FAM |
| ② | LEG Reaction Mix A2 | *NTRK1* Fusions | 35 μL | FAM |
| ③ | LEG Reaction Mix A3 | *NTRK2* Fusions | 35 μL | FAM |
| ④ | LEG Reaction Mix A4 | *NTRK3* Fusions & *HPRT1* | 35 μL | FAM, VIC |
| ⑤ | LEG Reaction Mix A5 | *ROS1* Fusions | 35 μL | FAM |
| ⑥ | LEG Reaction Mix A6 | *ROS1* Fusions | 35 μL | FAM |
| ⑦ | LEG Reaction Mix A7 | *MET* exon 14 skipping mutation | 35 μL | FAM |
| ⑧ | LEG Reaction Mix A8 | *RET* Fusions & *HPRT1* | 35 μL | FAM, VIC |

Table 3 Information of **LEG Reaction Mix B**

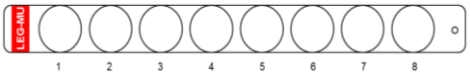
| Tube No. | Reagent | Target to detect | Quantity | Florescence Signal |
|---|---|---|---|---|
| ① | LEG Reaction Mix B1 | *EGFR* Mutations | 35 μL | FAM, VIC |
| ② | LEG Reaction Mix B2 | *EGFR* Mutations | 35 μL | FAM, VIC |
| ③ | LEG Reaction Mix B3 | *EGFR* Mutations | 35 μL | FAM, VIC |
| ④ | LEG Reaction Mix B4 | *EGFR/HER2* Mutations | 35 μL | FAM, VIC |
| ⑤ | LEG Reaction Mix B5 | *EGFR/KRAS* Mutations | 35 μL | FAM, VIC |
| ⑥ | LEG Reaction Mix B6 | *KRAS/HER2* Mutations | 35 μL | FAM, VIC |
| ⑦ | LEG Reaction Mix B7 | *KRAS/BRAF/EGFR* Mutations | 35 μL | FAM, VIC, ROX |
| ⑧ | LEG Reaction Mix B8 | External Control | 35 μL | FAM, VIC, ROX |

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

*For LEG Reaction Mix A:*




*For LEG Reaction Mix B:*




## Storage and Stability

The kit requires shipment on frozen ice packs. All components 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 PCR instruments: QuantStudio 5 or QuantStudio Dx.
2) DNA/RNA extraction kit: we recommend to use AmoyDx extraction kit (AmoyDx® FFPE DNA/RNA Kit for FFPE tumor tissue, Cat No.: 8.02.23601X036G, or AmoyDx® Tissue DNA Kit (Cat No.: 8.02.24301X036G), or AmoyDx® Tissue RNA Kit (Cat No.: 8.02.24601X036G) for fresh tumor tissue.)
3) Spectrophotometer for measuring DNA/RNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Nuclease-free PCR tubes and caps.
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

**For *in vitro* diagnostic use.**

### Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. Strictly follow the instructions during operation.
- Please check the compatibility of the real-time PCR instruments with the test kit prior to usage.
- 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.
- As all the chemicals have potential hazard, only trained professionals should 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 DNA/RNA 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 re-use.
- The unused reagents, used kit, and waste must be disposed of properly.

### Cleaning

- After the operation, 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 QuantStudio instruments, please set up as follows: Reporter Dye: FAM, VIC, ROX; Quencher Dye: None; Passive Reference: None.
- Refer to the operations manual of the real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use 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 tumor tissue samples. DNA/RNA extraction reagents are not included in the kit. Before the extraction of DNA and RNA it is very important to make sure that there is at least 30% tumor cells in the tumor tissue samples.

The $OD_{260/280}$ value of extracted DNA and RNA should be between 1.8~2.1.

The total RNA concentration for gene fusion detection is shown in Table 4.

Table 4 Recommended RNA concentration

| Sample type | Storage time | RNA concentration | Remark |
|---|---|---|---|
| FFPE tissue | ≤ 2 years | 10~100 ng/μL | • If RNA is between 10~100 ng/μL, use the original RNA without dilution;<br>• If RNA is more than 100 ng/μL, dilute the RNA to 100 ng/μL. |
| Fresh tissue | / | 2~30 ng/μL | • If RNA is between 2~30 ng/μL, use the original RNA without dilution;<br>• If RNA is more than 30 ng/μL, dilute the RNA to 30 ng/μL. |

The amount of extracted DNA for gene mutation detection is shown in Table 5.

Table 5 Recommended DNA concentration

| Sample type | Storage time | DNA concentration | DNA amount/reaction |
|---|---|---|---|
| FFPE tissue | ≤ 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 |
| Fresh tissue | / | 0.5~1 ng/μL | 2.5~5 ng |

***Note:***

- *The FFPE tissue should be handled and stored properly, and the storage time should preferably be less than 2 years.*
- *The extracted DNA and RNA should be used immediately. If not, it should be stored at -20±5℃. Avoid using extracted RNA stored for more than 2 weeks.*
- *The extracted DNA/RNA shall be measured by the spectrophotometer; the NanoDrop 1000 /2000 spectrophotometer is recommended.*
- *Before detection dilute the extracted DNA with 1×TE buffer (pH 8.0) to designated concentrations. Dilute the extracted RNA with nuclease-free water to designated concentration. We recommend using at least 5 μL DNA for 10 times dilution, to ensure the validity of final concentration.*

### 2. RNA Reverse Transcription

1) Take **LEG RT Reaction Mix I, LEG RT Reaction Mix II** and **LEG Reverse Transcriptase** out of the kit from the freezer. The other reagents should remain in freezer at -20±5℃.

2) Thaw the **LEG RT Reaction Mix I** and **LEG RT Reaction Mix II** at room temperature. When the reagents are completely thawed, invert each tube 10 times and briefly centrifuge to collect all liquid at the bottom of the tube.

3) Briefly centrifuge **LEG Reverse Transcriptase** prior to use.

4) For each RNA sample, prepare RNA reverse transcription solutions containing LEG Reverse Transcriptase, Sample RNA, and RT Reaction Mix (**LEG RT Reaction Mix I** or **LEG RT Reaction Mix II**, respectively) in separate 0.2 mL PCR tubes according to the ratio in Table 6. Thoroughly mix each reverse transcription solution by gently pipetting up and down more than 10 times.

Table 6 RNA Reverse Transcription Solutions

| Reagent | Volume per test |
|---|---|
| LEG RT Reaction Mix | 18.5 μL |
| LEG Reverse Transcriptase | 0.5 μL |
| Sample RNA | 6 μL |
| **Total** | **25 μL** |

5) Incubate the tubes at 42℃ for one hour.

6) Heat the tubes at 95℃ for 5 minutes, then transfer the PCR tubes on the ice. The resulting **Sample cDNA** is ready for PCR amplification. Mark the solutions as S-cDNA 1 and S-cDNA 2, (if more samples, name as S1-cDNA 1, S2-cDNA 1, …, Sn-cDNA 1 and S1-cDNA 2, S2-cDNA 2, …, Sn-cDNA 2)

***Note:*** *sample cDNA should be used immediately. If not, it should be stored at -20±5℃ for no more than one week after reverse transcription.*

## 3. RNA and DNA Mutations Detection

***Note:***

- *Each PCR run must contain one Positive Control (PC) and one no-template control (NTC).*
- *Do not vortex enzyme mix or any mixture with enzyme mix.*
- *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.*

1) Take out the **LEG PC** and thaw the reagents at room temperature. When the reagents are completely thawed, invert each tube for 10 times and briefly centrifuge to collect all liquid at the bottom of the tube.

2) Take out the **LEG Enzyme Mix A** and **LEG Enzyme Mix B**, briefly centrifuge prior to use.

3) Take out the sample cDNA, sample DNA, and nuclease-free water for NTC.

4) For **RNA detection**:

   a) Prepare S-Mix A1 and S-Mix A2: Add 1.3 μL **LEG Enzyme Mix** A into the above 25 μL **S cDNA 1** and **S cDNA 2** tube respectively. Mark the solutions as S-Mix A1 and S-Mix A2. Mix each solution thoroughly by pipetting up and down more than 10 times, and then centrifuge briefly.

   b) Prepare N-Mix A and P-Mix A: Add 2.34 μL **LEG Enzyme Mix** A into 45 μL nuclease-free water and 45 μL **LEG PC**, respectively. Mark the solutions as N-Mix A and P-Mix A. Mix each solution thoroughly by pipetting up and down more than 10 times, and then centrifuge briefly.

   c) Take out **LEG Reaction Mix A** strips (sufficient for samples, PC and NTC) and centrifuge the strips. Then gently uncover the caps prior to use.

   d) Prepare one **LEG Reaction Mix A** strip for NTC: Add 5 μL N-Mix A into Tube ①~⑧, cap the PCR tubes.

   e) Prepare one **LEG Reaction Mix A** strip for each sample: Add 5 μL S-Mix A1 into Tube ①~④, 5 μL S-Mix A2 into Tube ⑤~⑧, Cap the PCR tubes.

   f) Prepare one **LEG Reaction Mix A** strip for PC: Add 5 μL P-Mix A into Tube ①~⑧, cap the PCR tubes.

5) For **DNA detection**:

   a) Prepare LEG Master Mix B: Add 2.7 μL **LEG Enzyme Mix B** into 45 μL sample DNA/45 μL nuclease-free water/45 μL **LEG PC**, respectively. Mark the solutions as S-Mix B (if more samples, name as S1-Mix B, S2-Mix B, …, Sn-Mix B), N-Mix B,

P-Mix B. Mix each solution thoroughly by pipetting up and down more than 10 times, and centrifuge briefly.

b) Take out **LEG Reaction Mix B** strips (sufficient for samples, PC and NTC) and centrifuge the strips. Then gently uncover the caps prior to use.

c) Prepare one **LEG Reaction Mix B** strip for NTC: Add 5 µL N-Mix B into Tube ①~⑧, and cap the PCR tubes.

d) Prepare one **LEG Reaction Mix B** strip for each sample: Add 5 µL S-Mix B into Tube ①~⑧, and cap the PCR tubes.

e) Prepare one **LEG Reaction Mix B** strip for PC: Add 5 µL P-Mix B to Tube ①~⑧, and cap the PCR tubes.

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

7) Place the PCR tubes into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 7.

Table 7 Suggested PCR Plate Layout

| | RNA Detection | | | | | | DNA Detection | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Well** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **B** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **C** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **D** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **E** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **F** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **G** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |
| **H** | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC | Sample1 | Sample2 | Sample3 | Sample4 | NTC | PC |

8) Setup the PCR Protocol using the cycling parameters in Table 8.

Table 8 Cycling Parameters

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

9) Start the PCR run immediately.

10) When the PCR run is finished, analyze the data according to the "Results Interpretation" procedures.

## 4. Result Interpretation

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

1) For the NTC): The FAM Ct values of LEG Reaction Mix A1~A8, the FAM and VIC Ct values of LEG Reaction Mix B1~B7, and the ROX Ct value of LEG Reaction Mix B7 should be ≥ 36. If not, the data is *INVALID*. The sample should be retested.

2) For PC: The FAM Ct values of LEG Reaction Mix A1~A8 and VIC Ct values of LEG Reaction Mix A4/A8, the FAM and VIC Ct values of LEG Reaction Mix B1~B8, and the ROX Ct values of LEG Reaction Mix B7/B8 should be < 25. If not, the data is *INVALID*. The sample should be retested.

***Note:***

- *Select one reaction mix and one fluorescence channel at a time for fusion / mutation analysis.*
- *If there is low fluorescent signal, please zoom in the amplification curve.*

***Analyze RNA fusion assay for each sample:***

3) For LEG Reaction Mix A1~A8, analyze *ALK*, *NTRK1*, *NTRK2*, *NTRK3*, *ROS1*, *MET*, and *RET* gene fusions status:

a. Check the RNA Internal control VIC signals of LEG Reaction Mix A4/A8 for each sample:

i. If both VIC Ct values of LEG Reaction Mix A4/A8 are < 33 and either one is < 27, continue with the analysis.

ii. If both VIC Ct values of LEG Reaction Mix A4/A8 are ≥ 27, which indicates the partial fragmentation or degradation of

RNA, or the presence of PCR inhibitors, or any error with experimental operation. The corresponding assay should be retested with increased or re-extracted RNA.

b. Check FAM signals of LEG Reaction Mix A1~A8 for RNA gene variants of each sample (see Table 9):

Table 9 Result Determination

| **LEG Reaction Mix A** | **A1** | **A2** | **A3** | **A4** | **A5** | **A6** | **A7** | **A8** | **Results** |
|---|---|---|---|---|---|---|---|---|---|
| **Detected Target** | *ALK* | *NTRK1* | *NTRK2* | *NTRK3* | *ROS1* | *ROS1* | *MET* | *RET* | |
| Positive Ct range | Ct<33 | Ct<30 | Ct<30 | Ct<30 | Ct<33 | Ct<33 | Ct<30 | Ct<33 | Positive |
| Negative Ct range | Ct≥33 | Ct≥30 | Ct≥30 | Ct≥30 | Ct≥33 | Ct≥33 | Ct≥30 | Ct≥33 | Negative or under the LOD* |

* LOD: limit of detection

i. If any FAM Ct values of LEG Reaction Mix A1~A8 is in Positive Ct range, the sample is determined as corresponding fusion positive.

ii. If all the FAM Ct values of LEG Reaction Mix A1~A8 are in Negative Ct range, the sample is determined as negative (No fusion detected) or under the LOD of the kit.

***Analyze DNA mutation assay for each sample:***

4) For LEG Reaction Mix B1~B8, analyze DNA gene mutations status:

a. Check FAM, VIC and ROX signals of LEG Reaction Mix B8 for each sample:

i. If FAM, VIC and ROX Ct values of LEG Reaction Mix B8 are ≥19 and ≤26, continue with the analysis.

ii. If FAM, VIC and ROX Ct values of LEG Reaction Mix B8 is <19, it indicates that the DNA is overloaded. The DNA amount should be reduced. If the mutation signals of LEG Reaction Mix B1~B7 are negative, the results are plausible.

iii. If FAM, VIC and ROX Ct values of LEG Reaction Mix B8 >26, it indicates the partial fragmentation or degradation of DNA or the presence of PCR inhibitors, or any error with experimental operation. The sample should be retested with increased or re-extracted DNA.

b. Check FAM, VIC and ROX signals of LEG Reaction Mix B1~B7 for each sample (see Table 10):

Table 10 Result Determination

| **LEG Reaction Mix B** | | **B1** | **B2** | **B3** | **B4** | **B5** | **B6** | **B7** | **Results** |
|---|---|---|---|---|---|---|---|---|---|
| **FAM** | Optimal Ct range | Ct<31 | Ct<31 | Ct<31 | Ct<31 | Ct<31 | Ct<31 | Ct<31 | Positive |
| | Acceptable Ct range | 31≤Ct <34 | 31≤Ct <34 | 31≤Ct <33 | 31≤Ct <33 | 31≤Ct <33 | 31≤Ct <34 | 31≤Ct <34 | Interpret the results according to the ΔCt value |
| | ΔCt Cut-off value | 11 | 10 | 7 | 8 | 8 | 9 | 9 | |
| | Negative Ct range | Ct≥34 | Ct≥34 | Ct≥33 | Ct≥33 | Ct≥33 | Ct≥34 | Ct≥34 | Negative |
| **VIC** | Optimal Ct range | Ct<31 | Ct<31 | Ct<31 | Ct<31 | Ct<30 | Ct<30 | Ct<30 | Positive |
| | Acceptable Ct range | 31≤Ct <34 | 31≤Ct <33 | 31≤Ct <34 | 31≤Ct <34 | 30≤Ct <33 | 30≤Ct <33 | 30≤Ct <33 | Interpret the results according to the ΔCt value |
| | ΔCt Cut-off value | 8 | 8 | 9 | 9 | 9 | 8 | 9 | |
| | Negative Ct range | Ct≥34 | Ct≥33 | Ct≥34 | Ct≥34 | Ct≥33 | Ct≥33 | Ct≥33 | Negative |
| **ROX** | Optimal Ct range | / | / | / | / | / | / | Ct<30 | Positive |
| | Acceptable Ct range | / | / | / | / | / | / | 30≤Ct <33 | Interpret the results according to the ΔCt value |
| | ΔCt Cut-off value | / | / | / | / | / | / | 10 | |
| | Negative Ct range | / | / | / | / | / | / | Ct≥33 | Negative |

i. If any FAM/VIC Ct value of LEG Reaction Mix B1~B7 or ROX Ct value of LEG Reaction Mix B7 is in Optimal Ct range, the sample is determined as corresponding mutation positive.

ii. If any FAM/VIC Ct value of LEG Reaction Mix B1~B7 or ROX Ct value of LEG Reaction Mix B7 is in Acceptable Ct range, calculate the ΔCt value for each mutation showing positive amplification.

a) **ΔCt = Mutant FAM (VIC/ROX) Ct value - External Control FAM (VIC/ROX) Ct value**. The Mutant Ct value refers to FAM/VIC/ROX Ct value of sample mutant signal. External Control Ct value refers to FAM/VIC/ROX Ct value of the sample signal of external control.

b) If the ΔCt value is less than the corresponding cut-off ΔCt value, the sample is determined as positive (mutation detected).

c) If the ΔCt value is equal or more than the corresponding cut-off ΔCt value, the sample is determined as negative (no mutation detected) or under the LOD of the kit.

iii. If all the FAM and VIC Ct values of LEG Reaction Mix B1~B7, ROX Ct value of LEG Reaction Mix B7 are in Negative Ct range, the sample is determined as negative (no mutation detected) or under the LOD of the kit.

5) Some cross-reactivity may occur between *KRAS* mutation reactions. If the VIC signal in LEG Reaction Mix B5 and the FAM signal in LEG Reaction Mix B6 are both positive, the reaction mix with the smaller Ct value is determined as true positive, while the other reaction mix with bigger Ct value needs to be determined according to the cross-reactivity cut-off ΔCt value criteria (see Table 11).

a) If the ΔCt value is less than the cross-reactivity cut-off value, the positive curve is determined as true positive.

b) If the ΔCt value is greater than or equal to the cross-reactivity cut-off value, the result is determined as negative.

Table 11 Cross-reactivity Cut-off ΔCt value

| Reaction Mix / Signal / Mutation Name | B5/VIC | B6/FAM |
|---|---|---|
| KRAS-G12R (KRAS-M5) | 5.58 | |
| KRAS-G12C (KRAS-M6) | | 12.09 |

6) The sample may contain two or more variants simultaneously.

## Performance Characteristics

The performance characteristics of this kit were validated on QuantStudio 5 and QuantStudio Dx.

1) Sensitivity:
   - *For DNA mutation, the kit allows detection of an amount of 1~5% gene mutations in 10 ng DNA.*
   - *For RNA fusion, the kit allows detection of 25 copies/μL gene variant RNA.*
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:

   The accuracy of the kit was established by testing positive reference controls. The test gave positive results and positive concordance rate was 100%.
4) Precision: the precision of the kit was established by performing precision references for 10 repeats, all results were positive, the coefficient of variation for Ct values (CV, %) was less than 10%.
5) Interfering substance:

   Six potential interfering substances: hemoglobin, triglyceride, ethanol, xylene, proteinase K and paraffin were evaluated in this study. It is confirmed that 2 g/L hemoglobin, 37 mmol/L triglyceride, 21.7 mmol/L ethanol, 1% xylene, 0.1% high temperature denatured proteinase K, and 1% liquid paraffin would not interfere with the test result.

## Limitations

1) The kit is to be used only by personnel specially trained in the techniques of PCR and the use of real-time PCR instruments.
2) The results should not be the only criteria for diagnosis, they must be interpreted within the context of all relevant clinical and laboratory findings.
3) The kit has been validated for use with tumor tissue samples.
4) The kit can only detect the 167 hotspot variants listed in the appendix.
5) Reliable results are dependent on proper sample processing, transport, and storage.
6) If the sample only contains degraded DNA or RNA, this may affect the ability of the test to detect the intended mutations or fusions.

7) Samples with negative result (no mutation detected) may not mean that there are no mutations or fusions, but are just not detected by this assay.

## References

1) Hirsch, F. R. and P. A. Bunn, Jr. (2009). Lancet Oncol 10(5): 432-433.
2) Stephens, P., C. Hunter, et al. (2004). Nature 431(7008): 525-526.
3) Arcila, M. E., J. E. Chaft, et al. (2012). Clin Cancer Res 18(18): 4910-4918.
4) Li, M., L. Liu, et al. (2009). Oncol Rep 22(5): 1013-1020.
5) Renaud, S., J. Seitlinger, et al. (2017). J Thorac Dis 9(4): 957-960.
6) Del Re, M., E. Rofi, et al. (2018). Oncotarget 9(5): 6630-6643.
7) Planchard, D., B. Besse, et al. (2016). Lancet Oncol 17(7): 984-993.
8) Davies, H., G. R. Bignell, et al. (2002). Nature 417(6892): 949-954.
9) Perner, S., P. L. Wagner, et al. (2008). Neoplasia 10(3): 298-302.
10) Kwak, E. L., Y. J. Bang, et al. (2010). N Engl J Med 363(18): 1693-1703.
11) Paik, J. H., G. Choe, et al. (2011). J Thorac Oncol 6(3): 466-472.
12) Rodig, S. J., M. Mino-Kenudson, et al. (2009). Clin Cancer Res 15(16): 5216-5223.
13) Takeuchi, K., M. Soda, et al. (2012). Nat Med 18(3): 378-381.
14) Bergethon, K., A. T. Shaw, et al. (2012). J Clin Oncol 30(8): 863-870.
15) Cai, W., C. Su, et al. (2013). Cancer 119(8): 1486-1494.
16) Wang, R., H. Hu, et al. (2012). J Clin Oncol 30(35): 4352-4359.
17) Tsuta, K., T. Kohno, et al. (2014). Br J Cancer 110(6): 1571-1578.
18) Farago, A. F., M. S. Taylor, et al. (2018). JCO Precis Oncol 2018.
19) Liu, S. Y., L. Y. Gou, et al. (2016). J Thorac Oncol 11(9): 1503-1510.
20) Yang, J. C., Y. L. Wu, et al. (2015). Lancet Oncol 16(2): 141-151.
21) Mok, T. S., Y. L. Wu, et al. (2017). N Engl J Med 376(7): 629-640.
22) Solomon, B. J., T. Mok, et al. (2014). N Engl J Med 371(23): 2167-2177.
23) Peters, S., D. R. Camidge, et al. (2017). N Engl J Med 377(9): 829-838.
24) Shaw, A. T., S. H. Ou, et al. (2014). N Engl J Med 371(21): 1963-1971.
25) Drilon, A., S. Siena, et al. (2017). Cancer Discov 7(4): 400-409.
26) Drilon, A., T. W. Laetsch, et al. (2018). N Engl J Med 378(8): 731-739.
27) Farago.A, Kummar.S,et al. MA09.07 WCLC 2019.
28) Planchard, D., E. F. Smit, et al. (2017). Lancet Oncol 18(10): 1307-1316.
29) NCCN Clinical Practice Guidelines in Oncology: Non-Small Cell Lung Cancer. Version 7. 2019.
30) Drilon A, Wang L, Hasanovic A,et al. Cancer Discov 2013; 3: 630-635.
31) PROFILE1001. ASCO 2016. Abstract 108.
32) Li, B. T., R. Shen, et al. (2018). J Clin Oncol 36(24): 2532-2537.
33) Canon, J., K. Rex, et al. (2019). Nature 575(7781): 217-223.

## 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 |

# Appendix 1

**Gene Fusions Detected with LEG Reaction Mix A**

| Tube / Signal | Target to detect | Fusion Type | Name |
|---|---|---|---|
| ① FAM | *ALK* | *EML4* exon13;*ALK* exon20 | EML4-ALK-1 |
| | | *EML4* exon6 ins33;*ALK* exon20 | EML4-ALK-2 |
| | | *EML4* exon20;*ALK* exon20 | EML4-ALK-3 |
| | | *EML4* exon18;*ALK* exon20 | EML4-ALK-6 |
| | | *EML4* exon2;*ALK* exon20 | EML4-ALK-7 |
| | | *EML4* exon17;ins68 *ALK* exon20 | EML4-ALK-8 |
| | | *EML4* exon2;ins117 *ALK* exon20 | EML4-ALK-9 |
| | | *EML4* exon13;ins69 *ALK* exon20 | EML4-ALK-10 |
| | | *EML4* exon6;*ALK* exon20 | EML4-ALK-11 |
| | | *EML4* exon6;*ALK* exon19 | EML4-ALK-12 |
| | | *EML4* exon6;ins18 *ALK* exon20 | EML4-ALK-13 |
| | | *EML4* exon20;ins18 *ALK* exon20 | EML4-ALK-14 |
| | | *EML4* exon17del58;ins39 *ALK* exon20 | EML4-ALK-17 |
| | | *EML4* exon17 ins65;*ALK* exon20 | EML4-ALK-18 |
| | | *EML4* exon17;ins30 *ALK* exon20 | EML4-ALK-19 |
| | | *EML4* exon17 ins61;ins34 *ALK* exon20 | EML4-ALK-20 |
| | | *EML4* exon3;ins53 *ALK* exon20 | EML4-ALK-21 |
| | | *KIF5B* exon24;*ALK* exon20 | KIF5B-ALK-1 |
| | | *KIF5B* exon17;*ALK* exon20 | KIF5B-ALK-2 |
| | | *KLC1* exon9;*ALK* exon20 | KLC1-ALK |
| | | *TFG* exon4;*ALK* exon20 | TFG-ALK |
| ② FAM | *NTRK1* | *TFG* exon5;*NTRK1* exon9 | NTRK1-E9-M1 |
| | | *TPM3* exon8;*NTRK1* exon10 | NTRK1-E10-M1 |
| | | *SQSTM1* exon5;*NTRK1* exon10 | NTRK1-E10-M3 |
| | | *TPR* exon16 del54;*NTRK1* ins13 exon10 | NTRK1-E10-M5 |
| | | *TPR* exon21;*NTRK1* exon10 | NTRK1-E10-M6 |
| | | *CD74* exon8;*NTRK1* exon10 | NTRK1-E10-M7 |
| | | *IRF2BP2* exon1;*NTRK1* exon10 | NTRK1-E10-M8 |
| | | *IRF2BP2* exon1 del48;*NTRK1* exon10 | NTRK1-E10-M9 |
| | | *TFG* exon5;*NTRK1* exon10 | NTRK1-E10-M12 |
| | | *GRIPAP1* exon22;*NTRK1* exon10 | NTRK1-E10-M14 |
| | | *F11R* exon4;*NTRK1* exon10 | NTRK1-E10-M15 |
| | | *SQSTM1* exon6;*NTRK1* exon10 | NTRK1-E10-M17 |
| | | *TPM3* exon8;*NTRK1* exon12 | NTRK1-E12-M1 |
| | | *MPRIP* exon21;*NTRK1* exon12 | NTRK1-E12-M3 |
| | | *SSBP2* exon12;*NTRK1* exon12 | NTRK1-E12-M4 |
| | | *MPRIP* exon14;*NTRK1* exon12 | NTRK1-E12-M11 |
| | | *MPRIP* exon18;*NTRK1* exon12 | NTRK1-E12-M12 |
| | | *GRIPAP1* exon22;*NTRK1* exon12 | NTRK1-E12-M14 |
| ③ FAM | *NTRK2* | *TRIM24* exon12;*NTRK2* exon15 | NTRK2-E15-M1 |
| | | T*RIM24* exon12;*NTRK2* exon16 | NTRK2-E16-M1 |
| | | *SQSTM1* exon5;*NTRK2* exon16 | NTRK2-E16-M3 |
| | | *STRN* exon3;*NTRK2* exon16 | NTRK2-E16-M7 |
| | | *SQSTM1* exon5;*NTRK2* exon17 | NTRK2-E17-M2 |
| ④ FAM | *NTRK3* | *ETV6* exon4;*NTRK3* exon14 | NTRK3-EX14-M1 |
| | | *ETV6* exon5;*NTRK3* exon14 | NTRK3-EX14-M2 |
| | | *EML4* exon2;*NTRK3* exon14 | NTRK3-EX14-M3 |
| | | *SQSTM1* exon5;*NTRK3* exon14 | NTRK3-EX14-M4 |
| | | *RBPMS* exon5;*NTRK3* exon14 | NTRK3-EX14-M7 |
| | | *ETV6* exon5;*NTRK3* exon15 | NTRK3-EX15-M1 |
| | | *ETV6* exon4;*NTRK3* exon15 | NTRK3-EX15-M2 |
| | | *SQSTM1* exon6;*NTRK3* exon15 | NTRK3-EX15-M3 |
| ⑤ FAM | *ROS1* | *SLC34A2* exon4;*ROS1* exon32 | ROS1-M1 |
| | | *SLC34A2* exon13 del2046;*ROS1* exon32 | ROS1-M2 |
| | | *CD74* exon6;*ROS1* exon32 | ROS1-M3 |
| | | *SDC4* exon2;*ROS1* exon32 | ROS1-M4 |

| | | | |
|---|---|---|---|
| | | *SDC4* exon4;*ROS1* exon32 | ROS1-M5 |
| | | *SLC34A2* exon4;*ROS1* exon34 | ROS1-M6 |
| | | *SLC34A2* exon13 del2046;*ROS1* exon34 | ROS1-M7 |
| | | *CD74 exon6*;*ROS1* exon34 | ROS1-M8 |
| | | *SDC4* exon4;*ROS1* exon34 | ROS1-M9 |
| | | *EZR* exon10;*ROS1* exon34 | ROS1-M10 |
| ⑥ FAM | *ROS1* | *TPM3* exon8;*ROS1* exon35 | ROS1-M11 |
| | | *LRIG3* exon16;*ROS1* exon35 | ROS1-M12 |
| | | *GOPC* exon8;*ROS1* exon35 | ROS1-M13 |
| ⑦ FAM | *MET* | *MET* Exon 14 skipping mutation | MET-M1 |
| ⑧ FAM | *RET* | *CCDC6* exon1;*RET* exon12 | RET–M2 |
| | | *NCOA4* exon6;*RET* exon12 | RET–M5 |
| | | *KIF5B* exon15;*RET* exon12 | RET-M15 |
| | | *KIF5B* exon16;*RET* exon12 | RET–M16 |
| | | *KIF5B* exon23;*RET* exon12 | RET–M17 |
| | | *KIF5B* exon22;*RET* exon12 | RET–M19 |
| | | *TRIM33* exon14;*RET* exon12 | LRET-M22 |
| | | *CUX1* exon10;*RET* exon12 | LRET-M32 |
| | | *KIAA1468* exon10;*RET* exon12 | LRET-M40 |
| | | *KIF13A* exon18;*RET* exon12 | LRET-M41 |
| | | *MPRIP* exon19;*RET* exon12 | LRET-M42 |
| | | *MYO5C* exon25;*RET* exon12 | LRET-M44 |
| | | *PICALM* exon19;*RET* exon12 | LRET-M45 |
| | | *RUFY2* exon9;*RET* exon12 | LRET-M49 |
| | | *TNIP2* exon5;*RET* exon12 | LRET-M55 |
| | | *WAC* exon3;*RET* exon12 | LRET-M57 |

## Appendix 2

### Gene Mutations Detected with LEG Reaction Mix B

| Tube / Signal | Target to detect | Mutation | Base Change | cosmic ID | Name | LOD |
|---|---|---|---|---|---|---|
| ① FAM | *EGFR* Exon 19 | E746_A750del (1) | 2235_2249del15 | 6223 | E-19-M1 | 1% |
| | | E746_A750del (2) | 2236_2250del15 | 6225 | E-19-M2 | 1% |
| | | L747_P753>S | 2240_2257del18 | 12370 | E-19-M3 | 1% |
| | | E746_T751>I | 2235_2252>AAT(complex) | 13551 | E-19-M4 | 1% |
| | | E746_T751del | 2236_2253del18 | 12728 | E-19-M5 | 1% |
| | | E746_T751>A | 2237_2251del15 | 12678 | E-19-M6 | 1% |
| | | E746_S752>A | 2237_2254del18 | 12367 | E-19-M7 | 1% |
| | | E746_S752>V | 2237_2255>T(complex) | 12384 | E-19-M8 | 1% |
| | | E746_S752>D | 2238_2255del18 | 6220 | E-19-M9 | 1% |
| | | L747_A750>P | 2238_2248>GC(complex) | 12422 | E-19-M10 | 1% |
| | | L747_T751>Q | 2238_2252>GCA(complex) | 12419 | E-19-M11 | 1% |
| | | L747_E749del | 2239_2247del9 | 6218 | E-19-M12 | 1% |
| | | L747_T751del | 2239_2253del15 | 6254 | E-19-M13 | 1% |
| | | L747_S752del | 2239_2256del18 | 6255 | E-19-M14 | 1% |
| | | L747_A750>P | 2239_2248TTAAGAGAAG>C(complex) | 12382 | E-19-M15 | 1% |
| | | L747_P753>Q | 2239_2258>CA(complex) | 12387 | E-19-M16 | 1% |
| | | L747_T751>S | 2240_2251del12 | 6210 | E-19-M17 | 1% |
| | | L747_T751del | 2240_2254del15 | 12369 | E-19-M18 | 1% |
| | | L747_T751>P | 2239_2251>C(complex) | 12383 | E-19-M19 | 1% |
| | | L747_T751del | 2238_2252del15 | 23571 | E-19-M20 | 1% |
| | | L747_S752>Q | 2239_2256>CAA(Complex) | 12403 | E-19-M21 | 1% |
| | | L747_A750>P | 2239_2250>CCC(Complex) | / | E-19-M24 | 1% |
| | | L747_K754>QL | 2239_2261>CAATT(Complex) | / | E-19-M25 | 1% |
| | | E746_K754>EQHL | 2238_2261>GCAACATCT(Complex) | / | E-19-M26 | 1% |
| | | L747_S752>Q | 2238_2256>GCAA (Complex) | 26441 | E-19-M27 | 1% |
| ① VIC | *EGFR* Exon 20 | S768I | 2303G>T | 6241 | E-20-M2 | 1% |
| ② FAM | *EGFR* Exon 21 | L858R | 2573T>G | 6224 | E-21-M1 | 1% |
| ② VIC | *EGFR* Exon 18 | G719A | 2156G>C | 6239 | E-18-M1 | 1% |
| | | G719S | 2155G>A | 6252 | E-18-M2 | 2% |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | G719C | 2155G>T | 6253 | E-18-M3 | 1% |
| ③ FAM | *EGFR* Exon 20 | T790M | 2369C>T | 6240 | E-20-M1 | 2% |
| ③ VIC | *EGFR* Exon 21 | L861Q | 2582T>A | 6213 | E-21-M2 | 1% |
| ④ FAM | *EGFR* Exon 20 | H773_V774insH | 2319_2320insCAC | 12377 | E-20-M3 | 1% |
| | | D770_N771insG | 2310_2311insGGT | 12378 | E-20-M4 | 1% |
| | | V769_D770insASV | 2307_2308insGCCAGCGTG | 12376 | E-20-M5 | 1% |
| | | D770_N771insSVD | 2311_2312insGCGTGGACA | 13428 | E-20-M8 | 1% |
| | | V769_D770insASV | 2309_2310AC>CCAGCGTGGAT | 13558 | E-20-M9 | 5% |
| | | H773_V774insNPH | 2319_2320insAACCCCCAC | 12381 | E-20-M10 | 1% |
| | | .D770_N771insGF | 2310_2311insGGGTTT | 655155 | E-20-M14 | 1% |
| | | N771_P772insH | 2311_2312insACC | 6963572 | E-20-M16 | 1% |
| | | H773_V774insY | 2319_2320insTAC | / | E-20-M18 | 1% |
| | | H773_V774insPH | 2319_2320insCCCCAC | 12380 | E-20-M19 | 1% |
| | | V769_D770insGSV | 2308_2309insGCAGCGTGG | 18429 | E-20-M21 | 1% |
| | | N771_P772insHH | 2311_2312insACCACC | 6931207 | E-20-M22 | 1% |
| | | D770_N771insG | 2310_2311insGGG | / | E-20-M23 | 1% |
| | | D770_N771insG | 2310_2311insGGC | 13004 | E-20-M24 | 1% |
| | | P772_H773insDNP | 2307_2308insGACAACCCC | 6962050 | E-20-M26 | 1% |
| | | D770>GY | 2308_2309insGTT | 12427 | E-20-M34 | 1% |
| | | .D770_N771insGD | 2310_2311insGGGGAC | 85795 | E-20-M36 | 1% |
| | | D770_N771insGL | 2310_2311insGGGTTA | 48921 | E-20-M37 | 1% |
| | | N771>GF | 2311_2312AA>GGGTT | 18431 | E-20-M38 | 1% |
| | | N771_P772>SVDNR | 2312_2315ACCC>13(GCGTGGACAACCG) | 13554 | E-20-M40 | 1% |
| | | D770_N771insGT | 2310_2311insGGCACA | 1238029 | E-20-M41 | 1% |
| | | N771>KL | 2312_2313insACT | 6438147 | E-20-M44 | 2% |
| | | N771_P772insVDN | 2307_2308insGACAACGTG | 20885 | E-20-M52 | 1% |
| | | P772_H773insTP | 2316C>AACCCCT | 12388 | E-20-M55 | 1% |
| | | H773>PNPY | 2317_2318insCTAACCCCT | 1735761 | E-20-M56 | 1% |
| ④ VIC | *HER2* Exon 20 | G776>VC | 2326_2327insTGT | 12553 | HER2-M3 | 1% |
| | | P780_Y781insGSP | 2339_2340 insTGGCTCCCC | 303948 | HER2-M4 | 1% |
| | | P780_Y781insGSP | 2339_2340insGGGCTCCCC | 12555 | HER2-M6 | 1% |
| | | P780_Y781insGSP | 2340_2341insGGCTCCCCA | 12556 | HER2-M7 | 1% |
| | | G776>VC | 2326_2327insTTT | 12552 | HER2-M8 | 1% |
| | | P780_Y781insGSP | 2339_2340insCGGCTCCCC | 6865893 | HER2-M10 | 1% |
| | | G776>VC | 2326_2327insTAT | / | HER2-M15 | 1% |
| | | G776>VC | 2326_2327insTCT | 85995 | HER2-M16 | 1% |
| | | G776>LC | 2326G>TTAT | 20895 | HER2-M19 | 1% |
| | | G776>LC | 2326G>CTTT | 12554 | HER2-M20 | 1% |
| | | G776>LC | 2326G>TTGT | 19875 | HER2-M21 | 1% |
| | | V777_G778insCG | 2331_2332insTGTGGG | 303939 | HER2-M24 | 1% |
| ⑤ FAM | *EGFR* Exon 20 | V774_C775insHV | 2321_2322insCCACGT | 18432 | E-20-M32 | 1% |
| | | V774_C775insHV | 2322_2323insCACGTG | 22948 | E-20-M33 | 1% |
| | | H773_V774insAH | 2320_2321insCCCACG | 1238028 | E-20-M35 | 1% |
| | | P772_H773insV | 2316_2317insGTT | 255205 | E-20-M42 | 1% |
| ⑤ VIC | *KRAS* Exon 2 | G12C | 34G>T | 516 | KRAS-M6 | 1% |
| ⑥ FAM | *KRAS* Exon 2 | G12A | 35G>C | 522 | KRAS-M2 | 5% |
| | | G12V | 35G>T | 520 | KRAS-M3 | 2% |
| | | G12R | 34G>C | 518 | KRAS-M5 | 2% |
| | | G13C | 37G>T | 527 | KRAS-M14 | 1% |
| ⑥ VIC | *HER2* Exon 20 | A775_G776insYVMA | 2325_2326 ins12 (TACGTGATGGCT) | 12558 | HER2-M1 | 1% |
| | | A775_G776insYVMA | 2324_2325 ins12 (ATACGTGATGGC) | 20959 | HER2-M2 | 1% |
| ⑦ FAM | *KRAS* Exon 2 | G12D | 35G>A | 521 | KRAS-M1 | 5% |
| | | G12S | 34G>A | 517 | KRAS-M4 | 5% |
| ⑦ VIC | *BRAF* Exon 15 | V600E | 1799T>A | 476 | BRAF-M1 | 1% |
| ⑦ ROX | *EGFR* Exon 20 | C797S | 2389T>A | 6493937 | E-20-M6 | 1% |
| | | | 2390G>C | 5945664 | E-20-M7 | 1% |