AmoyDx
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CE IVD

# AmoyDx® *KRAS/NRAS* Mutations Detection Kit

Instructions for Use

| | | | |
|---|---|---|---|
| REF | 8.01.0030 | 6 tests/kit | For Stratagene Mx3000P™, ABI7500 |
| REF | 8.01.0031 | 6 tests/kit | For LightCycler480 |
| REF | 8.01.0032 | 6 tests/kit | For SLAN-96S |


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## Background

RAS protein is a GTPase and one of the key molecules in the downstream signaling pathway of epidermal growth factor receptor (EGFR). RAS protein transduces signals from membrane-bound receptors via multiple downstream effector pathways and thereby affects fundamental cellular processes, including proliferation, apoptosis, and differentiation. In total, activating *KRAS* and *NRAS* mutations occur in 20~50% and 1~6% of colorectal cancer respectively, mainly in exons 2, 3 or 4. The mutation status of the *RAS* gene is relevant to the primary drug resistance of colorectal cancers treated with anti-*EGFR* monoclonal antibodies. Patients with wild-type *KRAS* and *NRAS* gene could benefit from Erbitux (Cetuximab) or Vectibix (Panitumumab), whereas, the patients with mutant *KRAS* or *NRAS* gene show poor response to this treatment. RAS testing is recommended for the selection of colorectal cancer patients to receive anti-EGFR antibody therapy by NCCN Clinical Practice Guideline in Oncology and European Drug Administration Organization.

## Intended Use

The AmoyDx® *KRAS/NRAS* Mutations Detection Kit is a real-time PCR assay for qualitative detection of 19 *KRAS* mutations (exons 2, 3 and 4) and 13 *NRAS* mutations (exons 2, 3 and 4) in human genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue. The kit is intended to assess *KRAS/NRAS* mutation status in colorectal cancer 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

The kit adopts Amplification Refractory Mutation System (ARMS) and real-time PCR technologies, which comprises specific primers and probes to detect *KRAS*, *NRAS* mutations in human genomic DNA. The *KRAS*, *NRAS* mutant sequences are amplified by the mutant-specific primers, and detected by the fluorescence-labeled probes.

The kit is composed of 12 reaction mixes (KN Reaction Mix 1~11 and KN External Control Reaction Mix), sufficient positive control and enzyme mix.

1) The **KN Reaction Mix 1~11** includes mutation detection and internal control systems. The mutation detection system includes primers and FAM-labeled probes specific for designated *KRAS/NRAS* mutations, which is used to detect the *KRAS/NRAS* mutation status. The internal control system contains primers and HEX-labeled probe for a region of genomic DNA adjacent to the *RAS* gene, which is used to detect the presence of inhibitors and monitor the accuracy of the experimental operation.
2) The **KN External Control Reaction Mix** contains primers and FAM-labeled probe for a region of genomic DNA adjacent to the *RAS* gene, which is used to assess the quality of DNA.
3) The **KN Positive Control** (PC) contains recombinant gene with *KRAS* mutations and *NRAS* mutations.
4) The **KN Enzyme Mix** contains Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which is working 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 |
|---|---|---|
| **KN Reaction Mix** | 12-tube Strip* | 8 strips |
| **KN Enzyme Mix** | Taq DNA Polymerase, Uracil-N-Glycosylase | 40 μL/tube ×1 |
| **KN Positive Control** | Plasmid DNA | 250 μL/tube ×1 |

*Each strip (12-tube) includes the following contents for testing one sample or one control (see Table 2).

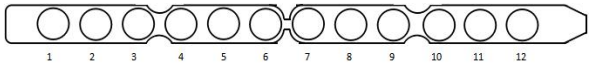
Table 2 Information of the 12-tube strip

| Tube No. | Reagent | Main Ingredients | Quantity | Fluorescent Signal |
|---|---|---|---|---|
| ① | **KN Reaction Mix 1** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ② | **KN Reaction Mix 2** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |

| | | | | |
|---|---|---|---|---|
| ③ | **KN Reaction Mix 3** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ④ | **KN Reaction Mix 4** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑤ | **KN Reaction Mix 5** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑥ | **KN Reaction Mix 6** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑦ | **KN Reaction Mix 7** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑧ | **KN Reaction Mix 8** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑨ | **KN Reaction Mix 9** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑩ | **KN Reaction Mix 10** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑪ | **KN Reaction Mix 11** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM, HEX/VIC |
| ⑫ | **KN External Control Reaction Mix** | Primers, Probes, $Mg^{2+}$, dNTPs | 35 μL | FAM |

***Note:***

*Distinguish Tube ⑫ from Tube ① according to the trapezoid end of strip edge, described as follows.*




## 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 eight months. The maximal number of freeze-thaw is five.

## Additional Reagents and Equipment Required but Not Supplied

1) Compatible Real-time PCR instruments:
   Stratagene Mx3000P™, ABI7500, LightCycler480 or SLAN-96S.
2) DNA extraction kit. We recommend to use AmoyDx DNA extraction kit (AmoyDx® FFPE DNA Kit) for paraffin embedded tissue specimens.
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. 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 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 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 instrument properly.
- For ABI instrument, please set up as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.
- For LightCycler480 instrument, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480. It's essential to place a 12-tube strip on the first row (Row A) of the adaptor.
- 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 real-time PCR instrument operator's manual 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 tumor tissue. The DNA extraction kit is 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 with *RAS* 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 ~ 2.0 (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.

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 extracted DNA should be used immediately. If not, it should be stored at -20±5℃ for no more than 6 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 **KN Positive Control (PC)**, and **KN Enzyme Mix** out of the kit from the freezer, and other reagents remained in freezer at -20±5℃.
2) Thaw the **KN Positive Control (PC)** at room temperature. When the reagent 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 **KN 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 No Template Control (NTC).
5) Prepare NTC mixture: pipet 65.8 μL nuclease-free water (NTC) and 4.2 μL KN Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.
6) Prepare sample DNA mixture: pipet 65.8 μL each sample DNA and 4.2 μL KN Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.
7) Prepare positive control (PC) mixture: pipet 65.8 μL KN Positive control and 4.2 μL KN Enzyme Mix into one centrifuge tube. Mix thoroughly by vortexing, and centrifuge for 5~10 seconds.

   ***Note:***

   - *Each 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.*
8) Take out the **KN Reaction Mix** (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 the NTC strip, and cap the PCR tubes.
10) Add 5 μL of prepared sample DNA mixture to each PCR tube of the sample strip, and cap the PCR tubes.
11) Add 5 μL of prepared PC mixture to each PCR tube of the 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 appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 PCR Plate Layout

| Assay / Well | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ | ⑨ | ⑩ | ⑪ | ⑫ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 | Sample 1 |
| **B** | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 | Sample 2 |
| **C** | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 | Sample 3 |
| **D** | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 | Sample 4 |
| **E** | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 | Sample 5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **F** | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 | Sample 6 |
| **G** | PC | PC | PC | PC | PC | PC | PC | PC | PC | PC | PC | PC |
| **H** | NTC | NTC | NTC | NTC | NTC | NTC | NTC | NTC | NTC | NTC | NTC | 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 ①~⑪ should be ≥31. If not, the data is *INVALID*. The sample should be retested.

2) For Positive Control: The FAM Ct values of Tubes ①~⑫ and HEX/VIC Ct values of Tubes ①~⑪ should be < 20. If not, the data is *INVALID*. The sample should be retested.

3) For the internal control assay in Tubes ①~⑪ for each sample: The HEX/VIC Ct values of Tubes ①~⑪ should be < 31. If not, check the FAM signals of Tubes ①~⑪:
   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 ⑫ for each sample:
   a) The FAM Ct value should be between 15~21.
   b) If the FAM Ct value is <15, this indicates the DNA is overloaded. The DNA amount should be reduced and retested. But if the FAM Ct values of Tubes ①~⑪ are in Negative Ct range (see Table 6), the sample is determined as negative.
   c) If the FAM Ct value is >21, 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 ①~⑪ is < 26, the sample is determined as positive.

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

5) Record the mutant FAM Ct values of Tubes ①~⑪ for each sample.

6) Check the mutant FAM Ct values of Tubes ①~⑪ according to Table 6:
   a) If any FAM Ct value of Tube ①~⑪ is < 26, the sample is determined as positive (*KRAS/NRAS* mutation detected).
   b) If any FAM Ct value of Tube ①~⑪ 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 less than the corresponding cut-off ΔCt value, the sample is determined as positive (Mutation detected).
      iii. 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 (Limit of Detection) of the kit.
   c) If all the FAM Ct values of Tubes ①~⑪ are in Negative Ct range or there is no amplification, the sample is determined as negative (No mutation detected) or under the LOD of the kit.

Table 6 Result Determination

| Mutation assay | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ | ⑨ | ⑩ | ⑪ | Results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Optimal Ct range | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Ct <26 | Positive. |
| Acceptable Ct range | 26≤Ct<28 | 26≤Ct<29 | 26≤Ct<29 | 26≤Ct<29 | 26≤Ct<29 | 26≤Ct<29 | 26≤Ct<28 | 26≤Ct<29 | 26≤Ct<29 | 26≤Ct<28 | 26≤Ct<29 | Interpret the results according to the ΔCt value. |
| Cut-off ΔCt value | 9 | 9 | 9 | 9 | 10 | 9 | 9 | 10 | 10 | 9 | 9 | |
| Negative Ct range | Ct≥28 | Ct≥29 | Ct≥29 | Ct≥29 | Ct≥29 | Ct≥29 | Ct≥28 | Ct≥29 | Ct≥29 | Ct≥28 | Ct≥29 | Negative or under the LOD*. |

* LOD: limit of detection

## Performance Characteristics

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

1) Analytical sensitivity:

   Analytical sensitivity of the kit was established using plasmid DNA. 32 single *KRAS/NRAS* mutant plasmid DNAs were diluted with 2 ng/μL wild-type DNA to prepare 32 plasmid DNAs of 1~5% mutant content. The 32 single *KRAS/NRAS* mutant plasmid DNAs of 1~5% mutant content were tested for 20 repeats using three batches of AmoyDx® *KRAS/NRAS* Mutations Detection Kit, and come out a positive rate of at least 95%. Therefore, the kit allows detection of 1~5% mutant DNA in a background of 95~99% normal DNA at 10 ng sample DNA amount.

   a. For Stratagene Mx3000P™: the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng DNA amount.

   b. For other PCR instrument: the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng DNA amount, except the LOD of NRAS-Q61R, Q61K and A146T mutations are 2% at 10 ng DNA amount, the LOD of KRAS-A146T and A146V are 5% at 10 ng DNA amount.

2) Specificity:

   The specificity of the kit was also evaluated 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:

   Three 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. Three lots of the kits were tested with the precision controls by two operators twice a day for 20 days on different PCR instruments. The Ct values were calculated, the CV values were all within 10%.

5) Cross-reactivity:

   The reaction with other homologous mutant nucleotide sequence (*HRAS* gene, as the same as *KRAS/NRAS* gene, is a member of RAS family, the plasmids with 3 *HRAS* hotspot mutations were selected in this study), and the cross reaction with non-human gene (the DNA was extracted from *Escherichia Coli, Yeast, Mycobacterium tuberculosis* and *streptococcus pneumonia* which were the common microorganism causing colorectal infection) were evaluated, the results showed no cross reactions.

6) Interfering substance:

   Three common potential interfering substances: hemoglobin, bilirubin and triglyceride, that easily cause abnormal physiological status, were evaluated in this study. It is confirmed that the potential maximum concentrations: 2 g/L hemoglobin, 15 g/L bilirubin 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 FFPE tumor tissue DNA.

4) The kit can only detect the 19 *KRAS* mutations and 13 *NRAS* 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 *KRAS/NRAS* mutation.
7) Samples with negative result (No mutation detected) may harbor *KRAS/NRAS* mutations not detected by this assay.

## References

1) Douillard JY, Oliner KS, Siena S, et al. 2013. Panitumumab-FOLFOX4 Treatment and RAS Mutations in Colorectal Cancer. The New England Journal of Medicine, 369 (11):1023-1034.
2) Di M, Pietrantonio F, Perrone F, et al. 2013. Lack of KRAS, NRAS, BRAF and TP53 mutations improves outcome of elderly metastatic colorectal cancer patients treated with cetuximab, oxaliplatin and UFT. Targeted Oncology, 1-8.
3) Roock WD, Claes Bart, Bernasconi D, et al. 2010. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol, 11: 753-762.

## 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

**KRAS and NRAS Mutations Detected by the Kit**

| Tube No. | Reagent | Exon | Mutation | Base change | Cosmic ID | Name |
|---|---|---|---|---|---|---|
| ① | KN Reaction Mix 1 | *KRAS* 2 | G12S | 34G>A | 517 | KRAS-M4 |
| | | | G12D | 35G>A | 521 | KRAS-M1 |
| ② | KN Reaction Mix 2 | *KRAS* 2 | G12C | 34G>T | 516 | KRAS-M6 |
| | | | G12R | 34G>C | 518 | KRAS-M5 |
| | | | G12V | 35G>T | 520 | KRAS-M3 |
| | | | G12A | 35G>C | 522 | KRAS-M2 |
| | | | G13C | 37G>T | 527 | KRAS-M14 |
| ③ | KN Reaction Mix 3 | *KRAS* 2 | G13D | 38G>A | 532 | KRAS-M7 |
| ④ | KN Reaction Mix 4 | *KRAS* 3 | A59T | 175G>A | 546 | KRAS-M25 |
| | | | Q61K | 181C>A | 549 | KRAS-M19 |
| ⑤ | KN Reaction Mix 5 | *KRAS* 3 | Q61L | 182A>T | 553 | KRAS-M15 |
| | | | Q61R | 182A>G | 552 | KRAS-M16 |
| | | | Q61H | 183A>C | 554 | KRAS-M17 |
| | | | Q61H | 183A>T | 555 | KRAS-M18 |
| ⑥ | KN Reaction Mix 6 | *KRAS* 4 | K117N | 351A>C | 19940 | KRAS-M20 |
| | | | K117N | 351A>T | 28519 | KRAS-M21 |
| | | | Al46T | 436G>A | 19404 | KRAS-M22 |
| | | | A146V | 437C>T | 19900 | KRAS-M23 |
| | | | A146P | 436G>C | 19905 | KRAS-M24 |
| ⑦ | KN Reaction Mix 7 | *NRAS* 2 | G12D | 35G>A | 564 | NRAS-M3 |
| | | | G12S | 34G>A | 563 | NRAS-M10 |
| ⑧ | KN Reaction Mix 8 | *NRAS* 2 | G13D | 38G>A | 573 | NRAS-M4 |
| ⑨ | KN Reaction Mix 9 | *NRAS* 2 | G13R | 37G>C | 569 | NRAS-M6 |
| | | | G12C | 34G>T | 562 | NRAS-M7 |
| | | | G12V | 35G>T | 566 | NRAS-M9 |
| | | | G12A | 35G>C | 565 | NRAS-M11 |
| | | | G13V | 38G>T | 574 | NRAS-M14 |
| ⑩ | KN Reaction Mix 10 | *NRAS* 3 | Q61R | 182A>G | 584 | NRAS-M1 |
| | | | Q61K | 181C>A | 580 | NRAS-M2 |
| | | | Q61L | 182A>T | 583 | NRAS-M5 |
| | | | Q61H | 183A>C | 586 | NRAS-M8 |
| ⑪ | KN Reaction Mix 11 | *NRAS* 4 | A146T | 436G>A | 27174 | NRAS-M12 |