AmoyDx
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CE IVD

AmoyDx® *KRAS* Mutation Detection Kit

Instructions for Use

REF 8.01.0128 24 tests/kit For Stratagene Mx3000P™, ABI7500, LightCycler480, Bio-Rad CFX96, 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

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Background

KRAS protein is a GTPase and one of the key molecules in the downstream signaling pathway of epidermal growth factor receptor (EGFR). KRAS 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 mutations in the *KRAS* genes occur in 15~30% of lung cancer and 20~50% of colorectal cancer, mainly in exons 2, 3 or 4. The mutation status of the *KRAS* gene is relevant to the primary drug resistance of colorectal cancers treated with anti-*EGFR* monoclonal antibodies. Patients with wild-type *KRAS* gene could benefit from Erbitux (Cetuximab) or Vectibix (Panitumumab), whereas, the patients with mutant *KRAS* gene show poor response to this treatment. The European Drug Administration Organization and US FDA approve the employment of *KRAS* gene mutation detection prior to the use of targeted medicines in the treatment of metastatic colorectal cancer.

Intended Use

The AmoyDx® *KRAS* Mutation Detection Kit is a real-time PCR assay for qualitative detection of 19 somatic mutations in codons 12, 13, 59, 61, 117 and 146 of *KRAS* gene in human genomic DNA extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue. The kit is intended to assess *KRAS* 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) 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 12 reaction mixes (KRAS^{19} Reaction Mix 1~11 and KRAS^{19} External Control Reaction Mix), sufficient positive control and enzyme mix.

1) The ***KRAS^{19}*** **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* mutations, which is used to detect the *KRAS* mutation status. The internal control system contains primers and HEX-labeled probe for a region of a region of genomic DNA adjacent to the *KRAS* gene, which is used to detect the presence of inhibitors and monitor the accuracy of the experimental operation.
2) The ***KRAS^{19}*** **External Control Reaction Mix** contains primers and FAM-labeled probe for a region of genomic DNA adjacent to the *KRAS* gene, which is used to assess the quality of DNA.
3) The ***KRAS^{19}*** **Positive Control (PC)** contains a recombinant gene with *KRAS* mutations.
4) The ***KRAS^{19}*** **Enzyme Mix** contains the 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

Tube No.	Content	Main Ingredients	Quantity	Fluorescent Signal
①	***KRAS^{19}*** **Reaction Mix 1**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
②	***KRAS^{19}*** **Reaction Mix 2**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
③	***KRAS^{19}*** **Reaction Mix 3**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
④	***KRAS^{19}*** **Reaction Mix 4**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
⑤	***KRAS^{19}*** **Reaction Mix 5**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
⑥	***KRAS^{19}*** **Reaction Mix 6**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
⑦	***KRAS^{19}*** **Reaction Mix 7**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
⑧	***KRAS^{19}*** **Reaction Mix 8**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC
⑨	***KRAS^{19}*** **Reaction Mix 9**	Primers, Probes, Mg^{2+}, dNTPs	1200 µL/tube ×1	FAM, HEX/VIC

⑩	***KRAS*^{I9} Reaction Mix 10**	Primers, Probes, Mg^{2+}, dNTPs	1200 μL/tube ×1	FAM, HEX/VIC
⑪	***KRAS*^{I9} Reaction Mix 11**	Primers, Probes, Mg^{2+}, dNTPs	1200 μL/tube ×1	FAM, HEX/VIC
⑫	***KRAS*^{I9} External Control Reaction Mix**	Primers, Probes, Mg^{2+}, dNTPs	1200 μL/tube ×1	FAM
/	***KRAS*^{I9} Enzyme Mix**	Taq DNA Polymerase, Uracil-N-Glycosylase	140 μL/tube ×1	/
/	***KRAS*^{I9} Positive Control**	Plasmid DNA	500 μL/tube ×2	/

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:
   Stratagene Mx3000P™, ABI7500, LightCycler480, Bio-Rad CFX96, 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) Nuclease-free PCR tubs and caps.
9) Adjustable pipettors and filtered pipette tips for handling DNA.
10) Tube racks.
11) Disposable powder-free gloves.
12) Sterile, nuclease-free water.
13) 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.
- As all the chemicals have potential hazard, 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 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.
- 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 tumor tissue. 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 *KRAS* 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 differs according to different storage time (see Table 2).

Table 2 Recommend 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) Thaw ***KRAS^{I9}* Reaction Mix 1~11**, ***KRAS^{I9}* External Control Reaction Mix** and ***KRAS^{I9}* PC** at room temperature. When the reagents are completely thawed, mix each reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect all liquid at the bottom of the tube.
2) Centrifuge ***KRAS^{I9}* Enzyme Mix** for 5~10 seconds prior to use.
3) Prepare sufficient *KRAS^{I9}* Master Mix 1~12 containing *KRAS^{I9}* Enzyme Mix and each *KRAS^{I9}* Reaction Mix (*KRAS^{I9}* Reaction Mix 1~11 or *KRAS^{I9}* External Control Reaction Mix, respectively) in separate sterile centrifuge tube according to the ratio in Table 3. Mix each *KRAS* Master Mix thoroughly by vortexing and centrifuge for 5~10 seconds.

Table 3 *KRAS^{I9}* Master Mix

Content	Volume per test
Reaction Mix	35 μL
***KRAS^{I9}* Enzyme Mix**	0.3 μL
Total volume	**35.3 μL**

Note:

- *Every PCR run must contain one PC (Positive control) 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.*

4) Take out the sample DNA (see Table 2 for DNA concentration) and nuclease-free water for NTC.
5) Prepare 12 PCR tubes for NTC: Dispense 35.3 μL of *KRAS^{I9}* Master Mix 1~12 to each PCR tube respectively. Then add 4.7 μL of nuclease-free water to each PCR tube, and cap the PCR tubes.
6) Prepare 12 PCR tubes for each sample: Dispense 35.3 μL of *KRAS^{I9}* Master Mix 1~12 to each PCR tube respectively. Then add 4.7 μL of sample DNA to each PCR tube, and cap the PCR tubes.
7) Prepare 12 PCR tubes for PC: Dispense 35.3 μL of *KRAS^{I9}* Master Mix 1~12 to each PCR tube respectively. Then add 4.7 μL of *KRAS^{I9}* Positive Control to each PCR tube, and cap the PCR tubes.
8) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
9) Place the PCR tubes into 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	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

10) Setup the PCR Protocol using the cycling parameters in Table 5.
11) Start the PCR run immediately.

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

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	/

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 13~21.
   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 ①~⑪ 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:

Table 6 Results Determination

Tube No.	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 <28	26≤Ct <29	26≤Ct <29	26≤Ct <28	26≤Ct <29	26≤Ct <29	26≤Ct <29	26≤Ct <29	26≤Ct <29	Interpret the results according to the ΔCt value.
Cut-off ΔCt value	9	10	11	10	9	10	9	10	9	10	9	
Negative Ct range	Ct≥28	Ct≥29	Ct≥28	Ct≥29	Ct≥29	Ct≥28	Ct≥29	Ct≥29	Ct≥29	Ct≥29	Ct≥29	Negative or under the LOD*.

* LOD: limit of detection

   a) If any FAM Ct value of Tube ①~⑪ is < 26, the sample is determined as positive (*KRAS* 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 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 (Limit of Detection) of the kit.

7) Some cross-reactivity may occur between *KRAS* mutation reactions. If a strong positive mutation is detected, some of the other mutation reactions may also give a positive curve. If two or more of the 19 mutations are detected, the one with the smallest Ct value is determined as true positive. Check the ΔCt value of other mutations to determine the result (see Table 7).
   a) If the ΔCt value is less than the cross-reactivity cut-off value, the positive curve is determined as true positive. A sample may be detected with one or more *KRAS* mutations.
   b) If the ΔCt value is greater than or equal to the cross-reactivity cut-off value, the result is determined as negative.

Table 7 Cross-reactivity Cut-off ΔCt value

Tube No. / Mutation Name	①	②	③	④	⑤	⑥	⑦	⑨	⑩
KRAS-M1		-	-	-	-	9.06	-	-	-
KRAS-M2	-		11.3	-	10.68	7.53	-	-	-
KRAS-M3	-	11.87		-	-	7.52	-	-	-
KRAS-M4	12.8	-	11.37		9.7	10.83	-	-	-
KRAS-M5	-	-	-	12.58		5.58	-	-	-
KRAS-M6	-	-	-	-	12.09		-	-	-
KRAS-M7	10.98	-	-	12.14	-	10.64		-	-
KRAS-M19	-	-	-	-	-	-	-		8.8
KRAS-M15	-	-	-	-	-	-	-	8.21	

Note: *"-" symbol of no cross-reactivity.*

Performance Characteristics

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

1) Limit of Detection:
   - For Stratagene Mx3000P™, the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng sample DNA amount.
   - For other PCR instruments, the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng sample DNA amount. Except: the sensitivity of *KRAS*-M16 and *KRAS*-M18 mutations are 2% at 10 ng sample DNA amount, the sensitivity of *KRAS*-M20, *KRAS*-M22 and *KRAS*-M23 mutations are 5% at 10 ng sample DNA amount.
2) Specificity:
   Specificity of the kit was established by testing 10 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 11 positive reference controls. The test gave positive results and positive concordance rate was 100%.
4) Interfering substance:
   4 common interfering substances: hemoglobin, triglyceride, *mycobacterium tuberculosis* and *streptococcus pneumonia* that are easily present in FFPE samples, were evaluated in this study. It is confirmed that the potential maximum concentrations: 2 mg/mL hemoglobin, 37 mmol/L triglyceride 10^6 CFU/mL *mycobacterium tuberculosis* and 10^6 CFU/mL *streptococcus pneumonia* would not interfere with the test result.
5) 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 10%.

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) Cross-reactivity was observed with *KRAS* G12F mutation in *KRAS*^{19} Reaction Mix 6, which accounts for ~0.48% lung cancer patients.
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* mutation.
7) Samples with negative result (No mutation detected) may harbor *KRAS* mutations not detected by this assay.

References

1) FDA website: http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm 172905.htm.
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3) Lièvre A, Bachet JB, Le Corre D, et al.2006. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 66 (8): 3992-5.
4) James RM, Arends MJ, Plowman SJ, et al.2003. KRAS Proto-Oncogene exhibits tumor suppressor activity as its absence promotes tumorigeneis in Murine Teratomas. Mol Cancer Res. 1: 820-5.
5) 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): 1023-34.
6) Roock WD, Jonker DJ, Nicolantonio FD, et al.2010. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA, 304(16): 1812-1820.

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* Mutations Detected by the Kit**

Tube No.	Reagent	Exon	Mutation	Base Change	Cosmic ID	Name
①	*KRAS*^{I9} Reaction Mix 1	2	G12D	35G>A	521	KRAS-M1
②	*KRAS*^{I9} Reaction Mix 2	2	G12A	35G>C	522	KRAS-M2
③	*KRAS*^{I9} Reaction Mix 3	2	G12V	35G>T	520	KRAS-M3
④	*KRAS*^{I9} Reaction Mix 4	2	G12S	34G>A	517	KRAS-M4
⑤	*KRAS*^{I9} Reaction Mix 5	2	G12R	34G>C	518	KRAS-M5
⑥	*KRAS*^{I9} Reaction Mix 6	2	G12C	34G>T	516	KRAS-M6
⑦	*KRAS*^{I9} Reaction Mix 7	2	G13D	38G>A	532	KRAS-M7
⑧	*KRAS*^{I9} Reaction Mix 8	2	G13C	37G>T	527	KRAS-M14
⑨	*KRAS*^{I9} Reaction Mix 9	3	A59T	175G>A	546	KRAS-M25
			Q61K	181C>A	549	KRAS-M19
⑩	*KRAS*^{I9} Reaction Mix 10	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
⑪	*KRAS*^{I9} Reaction Mix 11	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