



AmoyDx[®] *KRAS/NRAS/BRAF* Mutations Detection Kit

Detection of 17 *KRAS* mutations (exons 2, 3 and 4), 13 *NRAS* mutations (exons 2, 3 and 4)
and 6 *BRAF* V600 mutations (exon 15)

Instruction for Use

REF	8.01.25403W006A	6 tests	For Stratagene Mx3000P™, ABI7500
REF	8.01.25403W006B	6 tests	For LightCycler480
REF	8.01.25403W006D	6 tests	For SLAN-96S



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Background

KRAS, *NRAS* and *BRAF* gene are the key molecules in the downstream signaling pathway of epidermal growth factor receptor (EGFR), which transduce signals from membrane-bound receptors via multiple downstream effector pathways and thereby affect fundamental cellular processes, including proliferation, apoptosis, and differentiation. In total, activating *KRAS* and *NRAS* mutations occur in 20~50% and 1~6% of colorectal cancers respectively, mainly in exons 2, 3 or 4. And the most common *BRAF* mutations are V600 mutations in exon 15, which occur in 8~15% of colorectal cancers.

Studies have shown the mutation status of the *KRAS*, *NRAS* and *BRAF* 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. And *BRAF* gene mutations predict poor prognosis of such treatments. As a result, *KRAS*, *NRAS* and *BRAF* mutations detection supplies evidence for targeted clinical therapy of tumor patients, which decreases cost and time of treatment.

Intended Use

The AmoyDx® *KRAS/NRAS/BRAF* Mutations Detection Kit is a real-time PCR assay for qualitative detection of 17 *KRAS* mutations (exons 2, 3 and 4), 13 *NRAS* mutations (exons 2, 3 and 4) and 6 *BRAF* mutations (exon 15) in human DNA extracted from Formalin fix and Paraffin Embedded (FFPE) tissue. The kit is intended to be used to assess *KRAS/NRAS/BRAF* mutation status in colorectal cancer patient.

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

The kit is composed of 12 reaction mixes (KNB Reaction Mix 1~11 and KNB External Control Reaction Mix), Positive Control and Enzyme Mix.

- 1) The **KNB Reaction Mix 1~11** includes a mutation detection system and an internal control system. The mutation detection system contains primers and FAM-labeled probes specific for designated *KRAS/NRAS/BRAF* mutations, which is used to detect the mutation status of *KRAS*, *NRAS* and *BRAF* gene. The internal control system contains primers and HEX-labeled probe for a region of conserved segment in *RAS* gene, which is used to detect the presence of inhibitors and confirm the validity of each experiment.
- 2) The **KNB External Control Reaction Mix** contains primers and FAM-labeled probe for a region of genomic DNA adjacent to *KRAS*, *NRAS* and *BRAF* gene, which is used to assess the quality of DNA.
- 3) The **KNB Positive Control** contains recombinant gene with *KRAS* mutations, *NRAS* mutations and *BRAF* mutations.
- 4) The **KNB Enzyme Mix** contains Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.

Kit Contents

This kit contains the following materials (Table 1).

Table 1 Kit Contents

Content	Main Ingredients	Quantity
KNB Reaction Mix	12-tube Strip*	8 strips
KNB Enzyme Mix	Taq DNA Polymerase, Uracil-N-Glycosylase	40 µL
KNB Positive Control	Plasmid DNA	250 µL

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

Table 2 Information for 12-tube strip

Tube No.	Reagent	Main Ingredients	Quantity	Fluorescent Signal
①	KNB Reaction Mix 1	Primers, Probes, Mg ²⁺ , dNTPs,	35 µL	FAM, HEX/VIC

②	KNB Reaction Mix 2	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
③	KNB Reaction Mix 3	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
④	KNB Reaction Mix 4	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑤	KNB Reaction Mix 5	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑥	KNB Reaction Mix 6	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑦	KNB Reaction Mix 7	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑧	KNB Reaction Mix 8	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑨	KNB Reaction Mix 9	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑩	KNB Reaction Mix 10	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑪	KNB Reaction Mix 11	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM, HEX/VIC
⑫	KNB External Control Reaction Mix	Primers, Probes, Mg ²⁺ , dNTPs	35 μL	FAM

Note:

Distinguish Tube ⑫ 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°C and protected from light.

The shelf-life of the kit is eight months. The recommend maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

- Compatible real-time PCR instruments:
Stratagene Mx3000P™, ABI7500, LightCycler 480 or SLAN-96S.
- DNA extraction kit, we recommend use AmoyDx DNA Extraction Kit (AmoyDx® FFPE DNA Kit, Cat No.: 8.02.23501X036G).
- Spectrophotometer for measuring DNA concentration.
- Mini centrifuge with rotor for centrifuge tubes.
- Mini centrifuge with rotor for PCR tubes.
- Nuclease-free centrifuge tubes.
- Adjustable pipettors and filtered pipette tips for handling DNA.
- Tube racks.
- Disposable powder-free gloves.
- Sterile, nuclease-free water.
- 1×TE buffer (pH 8.0).

Precautions and Handling Requirements

For *in vitro* diagnostic use.

Precautions

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

Safety Information

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

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- 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.
- Using 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 μ L.
- For Stratagene Mx3000P™, if there's low net fluorescence signal (dR) but high background signals (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 I instrument, it's necessary to conduct fluorescence calibration prior to use. If there is fluorescence crossover on LightCycler480 II 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. 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 all PCR instruments in use should be conducted fluorescence calibration 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's essential to use standard pathology methodology to ensure tumor sample quality. Carry out the DNA extraction according to the instructions of DNA extraction kit.

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

The OD₂₆₀/OD₂₈₀ 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, and the storage time should preferably be less than 3 years.
- The extracted DNA should be used immediately, if not, it should be stored at $-20\pm 5^{\circ}\text{C}$ for no more than 6 months.
- Before detection, dilute the extracted tissue DNA with $1\times\text{TE}$ 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 **KNB Positive Control** and **KNB Enzyme Mix** out of the kit from the freezer, and other reagents remained in freezer at $-20\pm 5^{\circ}\text{C}$.
- 2) Thaw **KNB Positive Control** at room temperature. When the reagents completely thawed, invert the tube for 10 times and briefly centrifuge to collect all liquid at the bottom of the tube.
- 3) Briefly centrifuge **KNB Enzyme Mix** prior to use.
- 4) Take out the sample DNA (see Table 3 for DNA concentration) and nuclease-free water for NTC (No template control).
- 5) Prepare NTC mixture: pipet 65.8 μL nuclease-free water (NTC) and 4.2 μL **KNB Enzyme Mix** into one centrifuge tube. Mix each above mixture thoroughly by gently pipetting up and down more than 10 times.
- 6) Prepare sample DNA mixture: pipet 65.8 μL each sample DNA and 4.2 μL **KNB Enzyme Mix** into one centrifuge tube. Mix each above mixture thoroughly by gently pipetting up and down more than 10 times.
- 7) Prepare positive control (PC) mixture: pipet 65.8 μL **KNB Positive control** and 4.2 μL **KNB Enzyme Mix** into one centrifuge tube. Mix each above mixture thoroughly by gently pipetting up and down more than 10 times.

Note:

- Each run must contain one PC and one 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.
- 8) Centrifuge briefly.
 - 9) Take out the **KNB Reaction Mix** strips and centrifuge the strips if there are any reagent droplets in the caps of the PCR tubes. Then briefly uncover the caps prior to use.
 - 10) Add 5 μL of prepared NTC mixture to each PCR tube of the NTC strip, and cap the PCR tubes.
 - 11) Add 5 μL of each prepared sample DNA mixture to each PCR tube of the sample strip, and cap the PCR tubes.
 - 12) Add 5 μL of prepared PC mixture to each PCR tube of the PC strip, and cap the PCR tubes.
 - 13) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
 - 14) 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

15) Setup the PCR Protocol using the cycling conditions described in Table 5.

Table 5 Cycling Parameters

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

16) Start the PCR run immediately.

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

3. Results Interpretation

Before mutation data analysis, 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:

- 1) Check the mutant FAM Ct values of Tubes ①~⑪ for each sample.
- 2) 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/BRAF* mutations 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. $\Delta Ct \text{ value} = \text{Mutant FAM Ct value} - \text{External control FAM Ct value}$.
 - ii. If the ΔCt value is $<$ cut-off ΔCt value, the sample is determined as positive (Mutation detected).
 - iii. If the ΔCt value is \geq 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 of the kit).

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	$26 \leq Ct < 28$	$26 \leq Ct < 29$	$26 \leq Ct < 29$	$26 \leq Ct < 29$	$26 \leq Ct < 29$	$26 \leq Ct < 28$	$26 \leq Ct < 29$	$26 \leq Ct < 29$	$26 \leq Ct < 28$	$26 \leq Ct < 29$	$26 \leq Ct < 29$	Interpret the results according to the ΔCt value.
Cut-off ΔCt value	9	9	9	10	9	9	10	10	9	9	10	

Negative Ct range	Ct \geq 28	Ct \geq 29	Ct \geq 29	Ct \geq 29	Ct \geq 29	Ct \geq 28	Ct \geq 29	Ct \geq 29	Ct \geq 28	Ct \geq 29	Ct \geq 29	Negative or under the LOD*
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* LOD: limit of detection

Performance Characteristics

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

1. Sensitivity:

- (1) For Stratagene Mx3000P™, the kit allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng sample DNA amount.
- (2) 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, KRAS-M18, NRAS-M1, NRAS-M2 and NRAS-M12 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. Accuracy:

Accuracy of the kit was established by testing 36 mutant positive reference controls and 10 negative reference controls, the detection concordance rate are 100%.

3. Precision:

Precision of the kit was established by performing a certain mutant positive reference control for 10 repeats; all the controls can be detected.

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, combining 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 17 KRAS mutations and 13 NRAS mutations and 6 BRAF 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/BRAF mutation.
- 7) Samples with negative result (No mutation detected) may harbor KRAS/NRAS/BRAF 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. Rook 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



Authorized Representative in the European Community



In Vitro Diagnostic Medical Device



Manufacturer



Catalogue Number



Batch Code



Use By



Contains Sufficient for <n> Tests



Temperature Limitation



Consult Instructions For Use



Keep Dry



This Way Up



Fragile, Handle With Care

Appendix

KRAS, NRAS and BRAF Mutations Detected by the Kit

Tube No.	Reagent Supplied	Exon	Mutation	Base change	Cosmic ID	Name
①	KNB Reaction Mix 1	<i>KRAS</i> 2	G12S	34G>A	517	KRAS-M4
			G12D	35G>A	521	KRAS-M1
			G12C	34G>T	516	KRAS-M6
②	KNB Reaction Mix 2	<i>KRAS</i> 2	G12R	34G>C	518	KRAS-M5
			G12V	35G>T	520	KRAS-M3
			G12A	35G>C	522	KRAS-M2
③	KNB Reaction Mix 3	<i>KRAS</i> 2	G13C	37G>T	527	KRAS-M14
			G13D	38G>A	532	KRAS-M7
			Q61L	182A>T	553	KRAS-M15
④	KNB Reaction Mix 4	<i>KRAS</i> 3	Q61R	182A>G	552	KRAS-M16
			Q61H	183A>C	554	KRAS-M17
			Q61H	183A>T	555	KRAS-M18
⑤	KNB Reaction Mix 5	<i>KRAS</i> 4	K117N	351A>C	19940	KRAS-M20
			K117N	351A>T	28519	KRAS-M21
			A146T	436G>A	19404	KRAS-M22
			A146V	437C>T	19900	KRAS-M23
			A146P	436G>C	19905	KRAS-M24
⑥	KNB Reaction Mix 6	<i>NRAS</i> 2	G12D	35G>A	564	NRAS-M3
			G12S	34G>A	563	NRAS-M10
⑦	KNB Reaction Mix 7	<i>NRAS</i> 2	G13D	38G>A	573	NRAS-M4
			G13R	37G>C	569	NRAS-M6
			G12C	34G>T	562	NRAS-M7
			G12V	35G>T	566	NRAS-M9
			G12A	35G>C	565	NRAS-M11
⑧	KNB Reaction Mix 8	<i>NRAS</i> 2	G13V	38G>T	574	NRAS-M14
			Q61R	182A>G	584	NRAS-M1
			Q61K	181C>A	580	NRAS-M2
			Q61L	182A>T	583	NRAS-M5
⑨	KNB Reaction Mix 9	<i>NRAS</i> 3	Q61H	183A>C	586	NRAS-M8
			A146T	436G>A	27174	NRAS-M12
			V600E1	1799T>A	476	BRAF-M1
⑩	KNB Reaction Mix 10	<i>NRAS</i> 4	V600K	1798_1799GT>AA (complex)	473	BRAF-M2
			V600E2	1799_1800TG>AA (complex)	475	BRAF-M3
			V600R	1798_1799GT>AG (complex)	474	BRAF-M4
			V600D1	1799_1800TG>AC (complex)	\	BRAF-M5
			V600D2	1799_1800TG>AT (complex)	477	BRAF-M6
			V600D2	1799_1800TG>AT (complex)	477	BRAF-M6
⑪	KNB Reaction Mix 11	<i>BRAF</i> 15	V600D1	1799_1800TG>AC (complex)	\	BRAF-M5
			V600D2	1799_1800TG>AT (complex)	477	BRAF-M6
			V600D1	1799_1800TG>AC (complex)	\	BRAF-M5
			V600D2	1799_1800TG>AT (complex)	477	BRAF-M6
			V600D1	1799_1800TG>AC (complex)	\	BRAF-M5
			V600D2	1799_1800TG>AT (complex)	477	BRAF-M6