AmoyDx® KNBM PCR Panel

Instructions for Use

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

REF	8.01.0311	12 tests/kit	For LightCycler480 II, cobas z 480
	8.01.0312	12 tests/kit	For 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

Version: P1.0

Mar 2025

Background

Colorectal cancer is one of the most common and lethal cancers worldwide. According to the 2020 Global Cancer Statistics, it ranks third in incidence and second in mortality among 36 cancer types across 185 countries [1]. Colorectal cancer is associated with various genetic alterations, with the most common mutations in the KRAS, NRAS, and BRAF genes, as well as microsatellite instability (MSI). The frequency of KRAS mutations is approximately 32% [2], NRAS mutations about 4% [2], and BRAF mutations around 12% [2].

Microsatellite instability (MSI) is classified into three categories: microsatellite stable (MSS), microsatellite low instability (MSI-L), and microsatellite high instability (MSI-H). MSI-H is particularly significant in colorectal cancer, with the frequency of MSI-H varying by disease stage. MSI-H occurs in about 20% of patients with stage I and II colorectal cancer, around 12% in stage III, and 4-5% in stage IV colorectal cancer [3-4].

The NCCN Guidelines emphasize the critical role of genetic testing in guiding treatment for colorectal cancer. Patients without mutations in KRAS, NRAS, or BRAF may benefit from cetuximab or panitumumab therapies. Those with a BRAF p.V600E mutation can benefit from a combination of encorafenib with either cetuximab or panitumumab. Patients with KRAS p.G12C mutations may benefit from sotorasib in combination with cetuximab or panitumumab. MSI-H-positive patients can benefit from immune checkpoint inhibitors such as pembrolizumab or nivolumab. Therefore, comprehensive genetic testing is strongly recommended for colorectal cancer patients to guide treatment decisions and improve patient outcomes.

Intended Use

The AmoyDx® KNBM PCR Panel is a qualitative real-time PCR assay designed to detect 34 somatic mutations in KRAS gene (codons 12, 13, 59, 61, 117, and 146), 24 somatic mutations in NRAS gene (codons 12, 13, 59, 61, and 146), the BRAF V600E mutation and microsatellite instability (MSI) in formalin-fixed paraffin-embedded (FFPE) tissue samples from colorectal cancer patients. The kit is capable of genotyping specific mutations, including BRAF p.V600E, KRAS p.G12A/C/D/R/S/V, and KRAS p.G13D.

This kit is intended for research use only and must be operated by trained professionals in a controlled laboratory environment. The kit is compatible with the LightCycler480 II, cobas z 480 and SLAN-96S platforms for testing and data collection. Results should be analyzed by ARAS, a specialized tool designed to assist in the interpretation of the AmoyDx® KNBM PCR Panel results.

Principles of the Procedure

The AmoyDx® KNBM PCR Panel integrates advanced melting curve technology with real-time PCR, utilizing specific primers and multi-fluorescent channel probes (FAM, VIC, CY5) to detect a range of KRAS, NRAS and BRAF mutations, as well as MSI status in FFPE tissue samples from colorectal cancer patients. This approach significantly improves detection coverage and information yield.

The AmoyDx® KNBM PCR Panel includes **KNBM Reaction Mix** (12-tube strips), **KNBM Enzyme Mix**, and **KNBM Positive Control**. The kit is designed with 12-tube strips, each strip capable of testing one sample.

1) Tubes 1-3 of each strip contain reagents for evaluating MSI status and monitoring sample quality. The signals are indicated by FAM and CY5.

2) Tubes 4-7 of each strip contain reagents for detecting KRAS and NRAS gene mutations and monitoring sample quality. The signals are indicated by FAM and VIC.

3) Tube 8-12 of each strip contains reagents for detecting BRAF p.V600E and KRAS genotyped mutations along with internal control reagents. Mutation signals are indicated by FAM and VIC, while the internal control is indicated by CY5. The internal control targets a relatively conserved region of the human gene, used to monitor the sample quality and the PCR process. The internal control amplifies in all samples, regardless of the presence of mutations.

Additionally, the PCR amplification system in this kit contains UNG enzyme, which selectively cleaves the uracil-glycosidic bond in PCR fragments containing dU, effectively reducing false positives caused by PCR product contamination.

Kit Contents

This kit contains the following materials sufficient for up to 12 patient specimens per kit:

Table 1 Kit Contents

Content	Main Ingredients	Quantity
KNBM Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	8 strips ×2
KNBM Enzyme Mix	DNA Polymerase, Uracil-N-Glycosylase	350 µL/tube ×1
KNBM Positive Control	Plasmid DNA	400 µL ×1

Note:

- *Do not mix the reagent from different batches.*
- *The KNBM Positive Control contains a mixture of synthetic plasmid DNA sequences that correspond to a representative mutation per reaction detected by this kit.*

Table 2 Composition of the 12-tube Strip

Tube Number	Fluorescence	Target	Volume
1	FAM	EIF4E3	33.5 µL
	CY5	IFT140	
2	FAM	PRR5-ARHGAP8	33.5 µL
	CY5	PPP1CC	
3	FAM	TAOK3	33.5 µL
	CY5	ACVR2A	
4	FAM	KRAS codon 12 & 13	33.5 µL
5	FAM	KRAS codon 59 & 61	33.5 µL
	VIC	NRAS codon 12 & 13	
6	FAM	KRAS codon 117	33.5 µL
	VIC	NRAS codon 146	
7	FAM	KRAS codon 146	33.5 µL
	VIC	NRAS codon 59 & 61	
8	FAM	BRAF p.V600E	33.5 µL
	CY5	Internal Control	

9	FAM	KRAS p.G12D	33.5 μL
	VIC	KRAS p.G12C	
	CY5	Internal Control	
10	FAM	KRAS p.G12V	33.5 μL
	VIC	KRAS p.G12A	
	CY5	Internal Control	
11	FAM	KRAS p.G12S	33.5 μL
	VIC	KRAS p.G12R	
	CY5	Internal Control	
12	FAM	KRAS p.G13D	33.5 μL
	CY5	Internal Control	

Note:

- *Please distinguish Tube 12 from Tube 1 according to the trapezoid end of the strip edge, as described below.*

Storage and Stability

The kit requires shipment on frozen ice packs below 25℃ for no more than one week. All contents of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.

The shelf-life of the kit is twelve months. Tube opening doesn't affect the expiration of the kit. The recommended maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments: LightCycler480 II, cobas z 480, SLAN-96S
2) DNA extraction kit: AmoyDx® FFPE DNA Kit is recommended.
3) Spectrophotometers such as Nanodrop 1000/2000 are recommended 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

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 nucleic acid contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Assay Procedure

1. DNA Extraction

- The sample should be tumor FFPE tissue from a colorectal cancer patient fixed in 10% neutral formalin buffer. It is recommended to perform testing immediately after slicing the FFPE tissue sample.

- The FFPE tissue sample should contain a sufficient amount of tumor cells, with a recommended tumor content of 20% or higher. If the tumor content is lower, microdissection technology should be used for enrichment. The FFPE tissue sample should not be stored for more than 2 years.
- After DNA extraction, use a spectrophotometer to measure the concentration and purity of DNA. The DNA concentration should be greater than 5 ng/μL, and the $OD_{260/280}$ ratio should be between 1.5 and 2.3. Dilute the DNA with 1×TE (pH 8.0) buffer to a recommended concentration of 5 ng/μL for testing.
- It is recommended to test the extracted DNA immediately, otherwise it should be frozen and stored. During the storage period, avoid repeated freezing and thawing. The DNA should be stored at -20±5℃ for no more than 6 months.

2. KNBM Master Mix Preparation

In each PCR process, each sample must be tested and analyzed together with a Positive Control (PC) and a No Template Control (NTC, nuclease-free water).

1) Thaw the **KNBM Reaction Mix** (sufficient for samples as well as PC and NTC), **KNBM Positive Control**.
2) Vortex the above tubes to ensure no frozen components exist (5-10 seconds).
3) Spin the tubes briefly to collect the contents at the bottom of the tubes (5-10 seconds).
4) Place the above tubes into an ice rack.
5) Spin the **KNBM Enzyme Mix** briefly prior to use (5-10 seconds).
6) **KNBM Enzyme Mix** should be pre-mixed with samples (as well as control samples of NTC and PC) as a **KNBM Master Mix,** marked as S-Mix (if more samples, name as S1-Mix, S2-Mix, …, Sn-Mix), N-Mix, P-Mix. The ratio is as follows.

Table 3 KNBM Master Mix Composition

Reagent	Volume per test
Sample (Sample, PC, NTC)	65 μL
KNBM Enzyme Mix	19.5 μL

7) Mix the **KNBM Master Mix** by vortexing and briefly centrifuge (5-10 seconds each).

3. Plate Setup

Twelve reactions (Tubes 1-12) are set up for each sample. A single 96-well plate can accommodate up to 6 samples, one PC, and one NTC as seen in Figure 1 below.

1) Dispense 6.5 μL of the **KNBM Master Mix** into each tube of the 12-tube strip.
2) Seal the 12-tube strips with the optical sealing caps.
3) Briefly centrifuge (5-10 seconds) the strips to collect reaction at the bottom of the tubes and to ensure that there are no bubbles at the bottom of the tubes.
4) Place the **s**trips into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Table 4.

Table 4 AmoyDx® KNBM PCR Panel Layout for a Single Test Plate Analyzing 6 Unknown Samples

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8	Tube 9	Tube 10	Tube 11	Tube 12
S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1	S1
S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2	S2
S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3	S3
S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4	S4
S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5	S5
S6	S6	S6	S6	S6	S6	S6	S6	S6	S6	S6	S6
NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC
PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC	PC

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.*

4. Instrument Setup

Table 5 LightCycler480 II, cobas z 480 Real-Time PCR Parameters

Stage	Cycles	Temperature	Acquisition	Time	Ramp Rate	Acquistions	Analysis Mode
1	1	42℃	None	5 min	4.4℃	/	None
		95℃	None	5 min	4.4℃	/	
2	60	95℃	None	5 s	4.4℃	/	None
		60℃	None	15 s	2.2℃	/	
		72℃	None	10 s	4.4℃	/	
3	1	95℃	None	1 min	4.4℃	/	Melting Curves
		45℃	None	3 min	2.2℃	/	
		35℃	None	1 min	1.5℃	/	
4	1	35℃	Continuous	/	0.04℃	5	Melting Curves
		75℃	Continuous	/	0.04℃	5	
5	1	40℃	None	30 s	2.2℃	/	None

Note:

- *For LightCycler480 II, select 465-510 (FAM), 533-580(VIC), and 618-660(CY5) for "Filter Combination".*
- *For cobas z 480, select 465-510 (FAM), 540-580(VIC), and 610-670(CY5) for "Filter Combination".*

Table 6 SLAN-96S Real-Time PCR Parameters

Stage	Cycles	Temperature	Time	Data Collection
1	1	42℃	5 min	/
		95℃	5 min	/
2	60	95℃	5 s	/
		60℃	15 s	/
		72℃	10 s	/
3	1	95℃	1 min	/
		45℃	3 min	/
		35℃	1 min	/
4	1	35℃-75℃	/	Melting Curve
5	1	40℃	30 s	/

Note:

- *Select 470-510 (FAM), 530-565(VIC), and 630-665(CY5) for "Fluorophores/Dyes".*
- *In stage 4, please select "Continuous" and set "Ramp Rate" as 0.02℃/s.*

To complete the instrument setup, please follow the provided instructions to import the real-time PCR run configuration file. Ensure all parameters are correctly configured according to the file for optimal performance.

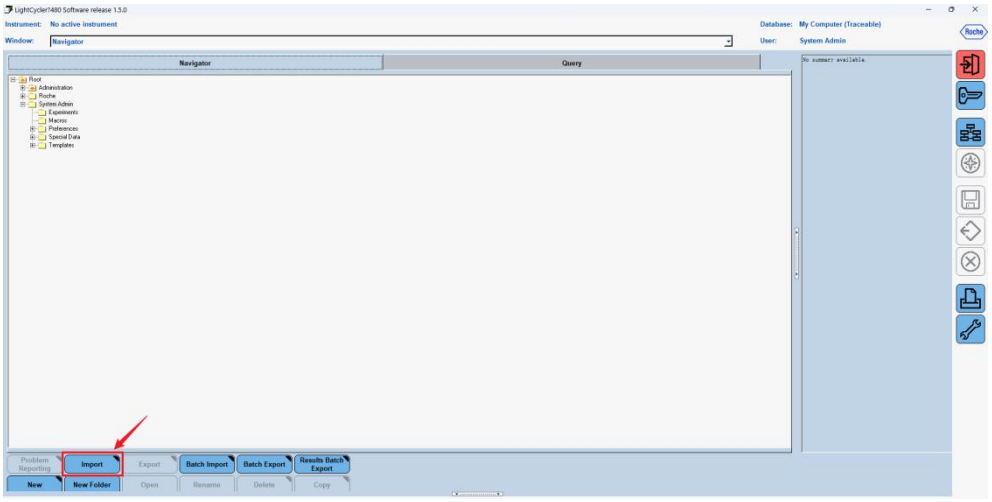
- LightCycler480 II or cobas z 480

1) Open the LightCycler480 software.

2) Click "New experiment" in the main page.

3) Select "Nevigator" in the drop-down list of "Window".

4) Click "Import" in the bottom bar.



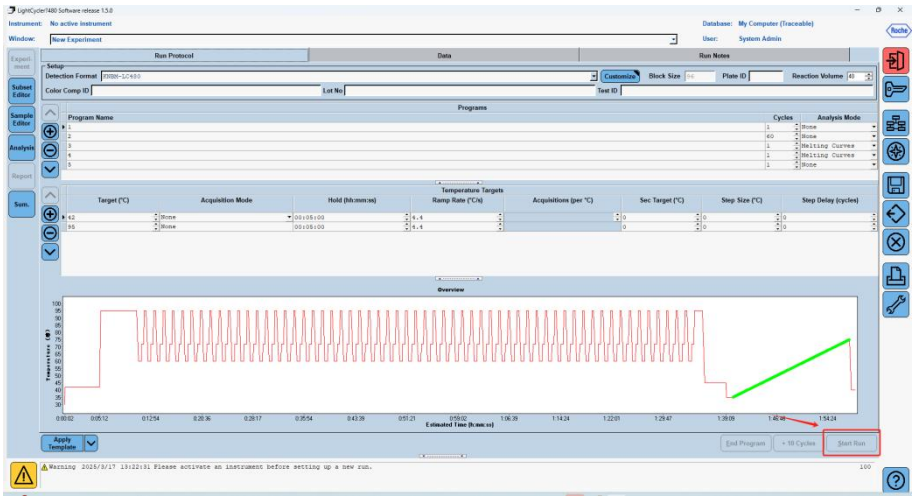
5) Select the appropriate AmoyDx KNBM PCR Panel run protocol template based on your instrument type:

   a) For LightCycler480 II, use "AmoyDx KNBM PCR Panel for LC480.ixo".

   b) For cobas z 480, use "AmoyDx KNBM PCR Panel for cobas z480.ixo".

If the project file is unavailable, contact your regional Field Application Scientist for assistance.

6) Click “Start Run” button to start the PCR process.



7) Click “Sample Editor” button on the left sidebar to enter sample information.

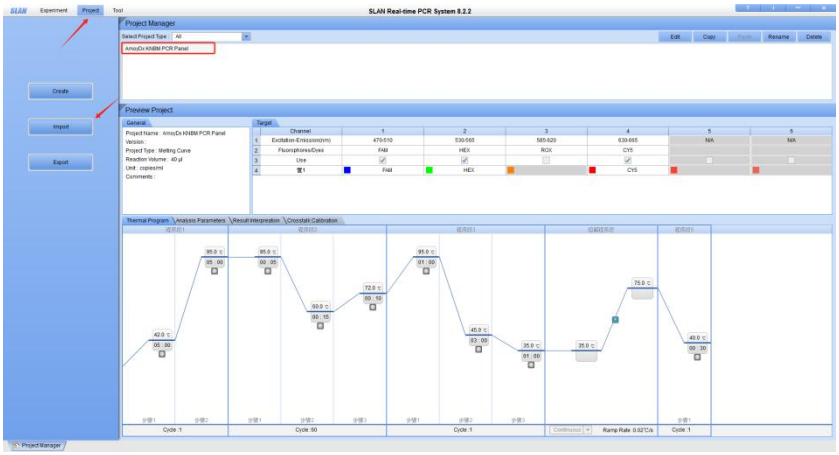
- SLAN-96S

1) Open the SLAN-96S software.

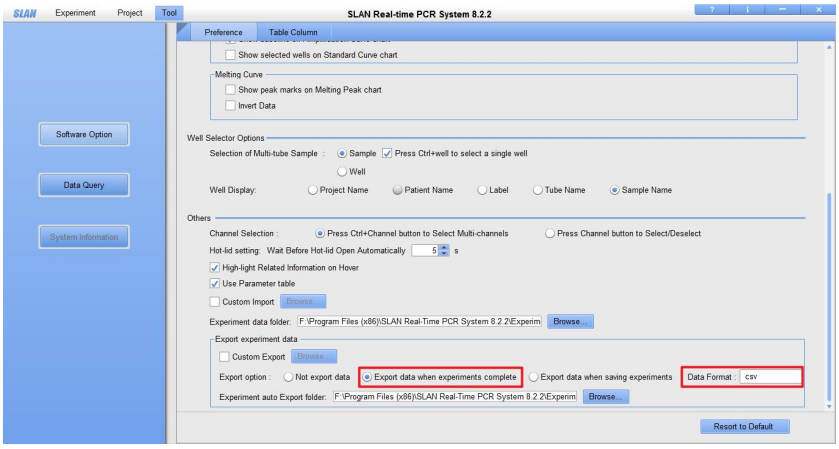
2) Click “Project” in the top bar.

3) Click “Import” in the left sidebar.

4) Select the run protocol template “AmoyDx KNBM PCR Panel.prj”. If the file is not available, please reach out to your regional Field Application Scientist.



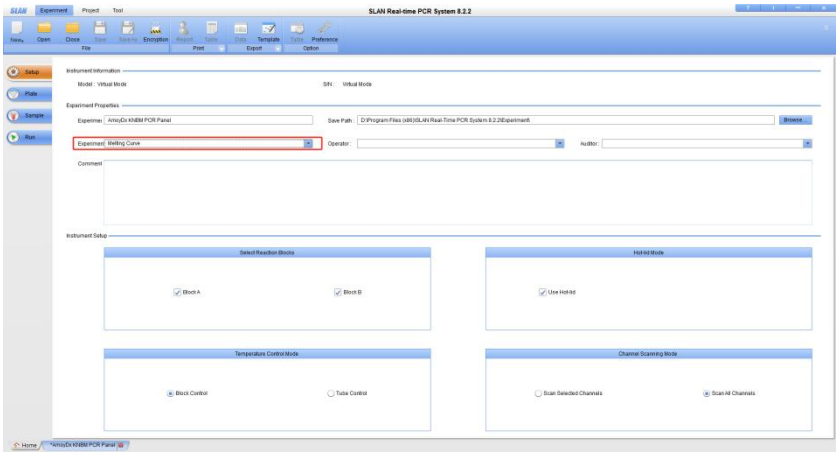
5) Click the “Tool” button and Select “Export data when experiments complete” and set the data format as “csv” for “Export option”.



6) Click “Experiment” button in the top bar.

7) Click “Experiment Wizard” in the left sidebar.

8) Select “Melting Curve” for the Experiment and set the other experiment properties per your request.

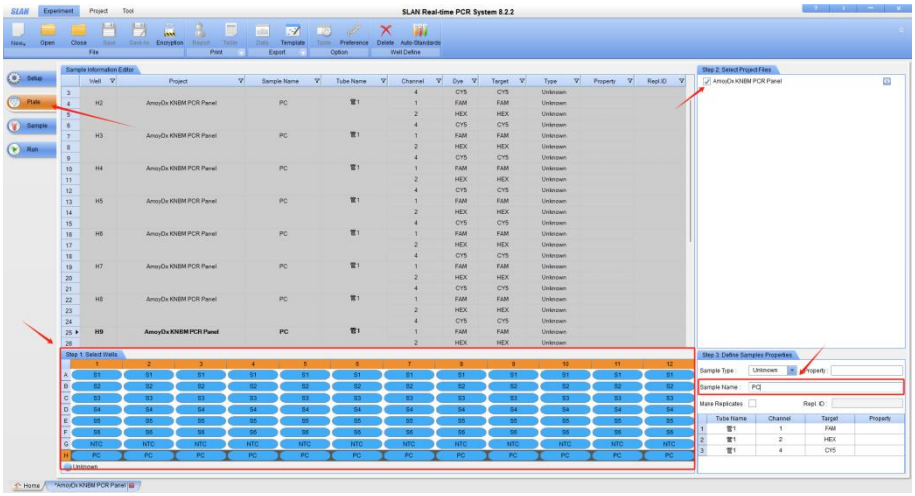


9) Click the “Plate” button on the left sidebar to set the plate information.

   a) Highlight the wells you assigned the PCR Strips.

   b) Select the project file “AmoyDx KNBM PCR Panel.prj”.

   c) Insert the sample names.



10) Click the “Run” button on the left sidebar and click “Start” to start the PCR process.

5. Result Interpretation

ARAS is a proprietary software developed by AmoyDx intended to be used as a tool to aid in the interpretation of AmoyDx® KNBM PCR Panel. Upon the PCR run completion, import the PCR data into the ARAS, to determine sample’s mutation status based on the melting peak of the target mutations.

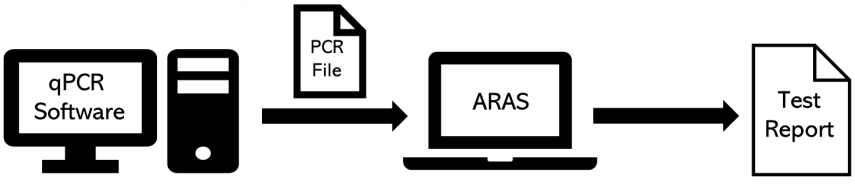


Figure 2 PCR Data to ARAS Workflow Overview

1) Enter ARAS's IP address 127.0.0.1 in the Chrome browser and provide their account credentials to access ARAS.
2) Click the "Create New Analysis" button and a pop-up window will appear, select the product and instrument you wish to analyze.
3) Click the upload button to select the PCR file to be analyzed and then click "Confirm" to initiate the analysis.
    a) PCR file from LightCycler480 II or cobas z 480 should be in zip format.
    b) PCR file from SLAN-96S should be in csv format.
4) Assign the sample layout based on the experiment, and then click the "Analysis" button to generate testing results.
5) On the result page, users can verify the accuracy of the test results by reviewing the result list, 96-well plate diagram and the amplified fluorescence curves.
6) On the report page, click the "Generate report" button to generate and download report files for the tested samples.

Note:

- *ARAS is For Research Use Only. Not for use in diagnostic procedures.*
- *PCR file generated from different instruments may necessitate distinct preparation before ARAS analysis. Please adhere the ARAS protocol to ensure that the PCR file is adequately interpretable by ARAS.*
- *For other functions provided by ARAS, please refer to the instructions of ARAS.*

Performance Characteristics

1) The kit's appearance is clean, with clear labeling and no leakage. After thawing, the reagents are clear, with no turbidity or precipitation.
2) Under the specified testing conditions, the kit's limit of detection for the hotspot mutations ranges from 1%-5% at a DNA input of 25 ng, for rare mutations, the limit of detections might be >5% (see Appendix for details).
3) Accuracy of the kit was established by testing internal positive references and negative references, the detection rates are 100%.

Limitations

1) This kit is intended for use only by individuals who have received specialized training in PCR techniques.
2) A negative result does not completely exclude the presence of a gene mutation. A negative result may occur if the tumor DNA in the sample is too scarce, severely degraded, or if the concentration of the DNA mutation in the amplification reaction system is below the detection limit.
3) Improper sample collection, transportation, handling, as well as incorrect experimental procedures and environmental conditions, may lead to false-negative or false-positive results.
4) This test is limited to the specified sample types and detection systems (including applicable instruments, nucleic acid extraction reagents, and detection methods).
5) This kit is only applicable for mutation detection of FFPE sample DNA and is not suitable for plasma sample DNA detection.
6) This panel covers the declared mutations (see Appendix) and may also detect additional mutation sites within the same codon in tubes 4–7.
7) The test results obtained with this panel are for research purposes only and should not be used for diagnostic procedures.

Reference

1) Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.

2) Guo L, Wang Y, Yang W, et al. Molecular Profiling Provides Clinical Insights Into Targeted and Immunotherapies as Well as Colorectal Cancer Prognosis. Gastroenterology 2023;165:414-28 e7.

3) Ashktorab H, Ahuja S, Kannan L, et al. A meta-analysis of MSI frequency and race in colorectal cancer. Oncotarget 2016;7:34546-57.

4) Battaglin F, Naseem M, Lenz HJ, Salem ME. Microsatellite instability in colorectal cancer: overview of its clinical significance and novel perspectives. Clin Adv Hematol Oncol 2018;16:735-45.

Symbols

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

Appendix

Tube	Fluorescence	Target	MUTATION_AA	MUTATION_CDS	Cosmic ID	Name	LOD
1	FAM	EIF4E3	/	$(A)_{12}\rightarrow(A)_{10}$	/	EIF4E3-M2	5%
	CY5	IFT140	/	$(T)_{11}\rightarrow(T)_{10}$	/	IFT140-M2	5%
2	FAM	PRR5-ARHGAP8	/	$(T)_{12}\rightarrow(T)_{10}$	/	ARHGAP8-M2	5%
	CY5	PPP1CC	/	$(A)_{12}\rightarrow(A)_{10}$	/	PPP1CC-M2	5%
3	FAM	TAOK3	/	$(A)_{12}\rightarrow(A)_{10}$	/	TAOK3-M2	5%
	CY5	ACVR2A	/	$(A)_{8}\rightarrow(A)_{7}$	/	ACVR2A-M2	5%
4	FAM	KRAS codon 12 and 13	p.G12D	c.35G>A	COSM521	KRAS-M1	5%
			p.G12A	c.35G>C	COSM522	KRAS-M2	5%
			p.G12V	c.35G>T	COSM520	KRAS-M3	5%
			p.G12S	c.34G>A	COSM517	KRAS-M4	5%
			p.G12R	c.34G>C	COSM518	KRAS-M5	5%
			p.G12C	c.34G>T	COSM516	KRAS-M6	5%
			p.G13D	c.38G>A	COSM532	KRAS-M7	5%
			p.G13C	c.37G>T	COSM527	KRAS-M14	5%
			p.G12F	c.34_35delinsTT	COSM512	KRAS-G12F-M1	5%
			p.G12I	c.34_35delinsAT	COSM34144	KRAS-G12I-M1	5%
			p.G12W	c.34_36delinsTGG	COSM36281	KRAS-G12W-M1	5%
			p.G13D	c.38_39delinsAT	COSM531	KRAS-G13D-M1	5%
			p.G13R	c.37G>C	COSM529	KRAS-G13R-M1	5%
			p.G13S	c.37G>A	COSM528	KRAS-G13S-M1	5%
			p.G13V	c.38G>T	COSM534	KRAS-G13V-M1	5%
			p.G13V	c.38_39delinsTT	COSM12721	KRAS-G13V-M2	5%
5	FAM	KRAS codon 59 and 61	p.Q61L	c.182A>T	COSM553	KRAS-M15	5%
			p.Q61R	c.182A>G	COSM552	KRAS-M16	5%
			p.Q61H	c.183A>C	COSM554	KRAS-M17	5%
			p.Q61H	c.183A>T	COSM555	KRAS-M18	5%
			p.Q61K	c.181C>A	COSM549	KRAS-M19	5%
			p.Q61E	c.181C>G	COSM550	KRAS-Q61E-M1	5%
			p.Q61K	c.180_181delinsAA	COSM87298	KRAS-Q61K-M1	5%
			p.Q61P	c.182A>C	COSM551	KRAS-Q61P-M1	5%
			p.A59T	c.175G>A	COSM546	KRAS-M25	5%
			p.A59E	c.176C>A	COSM547	KRAS-A59E-M1	20%
			p.A59G	c.176C>G	COSM28518	KRAS-A59G-M1	20%
	VIC	NRAS codon 12 and 13	p.G12D	c.35G>A	COSM564	NRAS-M3	5%
			p.G13D	c.38G>A	COSM573	NRAS-M4	5%
			p.G13R	c.37G>C	COSM569	NRAS-M6	5%
			p.G12C	c.34G>T	COSM562	NRAS-M7	5%
			p.G12V	c.35G>T	COSM566	NRAS-M9	5%
			p.G12S	c.34G>A	COSM563	NRAS-M10	5%
			p.G12A	c.35G>C	COSM565	NRAS-M11	5%
			p.G13C	c.37G>T	COSM570	NRAS-M13	5%
			p.G13V	c.38G>T	COSM574	NRAS-M14	5%
			p.G12R	c.34G>C	COSM561	NRAS-G12R-M1	5%
			p.G13A	c.38G>C	COSM575	NRAS-G13A-M1	5%
			p.G13S	c.37G>A	COSM571	NRAS-G13S-M1	5%

6	FAM	KRAS codon 117	p.K117N	c.351A>C	COSM19940	KRAS-M20	5%
			p.K117N	c.351A>T	COSM28519	KRAS-M21	5%
			p.K117R	c.350A>G	COSM469672	KRAS-K117R-M1	5%
			p.K117E	c.349A>G	COSM685442	KRAS-117E-M1	5%
	VIC	NRAS codon 146	p.A146T	c.436G>A	COSM27174	NRAS-M12	5%
			p.A146P	c.436G>C	COSM417257	NRAS-A146P-M1	20%
			p.A146V	c.437C>T	COSM417022	NRAS-A146V-M1	20%
7	FAM	KRAS codon 146	p.A146T	c.436G>A	COSM19404	KRAS-M22	5%
			p.A146V	c.437C>T	COSM19900	KRAS-M23	5%
			p.A146P	c.436G>C	COSM19905	KRAS-M24	5%
	VIC	NRAS codon 59 and 61	p.Q61R	c.182A>G	COSM584	NRAS-M1	5%
			p.Q61K	c.181C>A	COSM580	NRAS-M2	5%
			p.Q61L	c.182A>T	COSM583	NRAS-M5	5%
			p.Q61H	c.183A>C	COSM586	NRAS-M8	5%
			p.Q61H	c.183A>T	COSM585	NRAS-M18	5%
			p.Q61E	c.181C>G	COSM581	NRAS-Q61E-M1	5%
			p.Q61P	c.182A>C	COSM582	NRAS-Q61P-M1	5%
			p.A59T	c.175G>A	COSM578	NRAS-A59T-M1	5%
			p.A59D	c.176C>A	COSM253327	NRAS-M15	20%
8	FAM	BRAF codon 600	p.V600E	c.1799T>A	COSM476	BRAF-M1	1%
9	FAM	KRAS codon 12	p.G12D	c.35G>A	COSM521	KRAS-M1	1%
	VIC	KRAS codon 12	p.G12C	c.34G>T	COSM516	KRAS-M6	1%
10	FAM	KRAS codon 12	p.G12V	c.35G>T	COSM520	KRAS-M3	1%
	VIC	KRAS codon 12	p.G12A	c.35G>C	COSM522	KRAS-M2	1%
11	FAM	KRAS codon 12	p.G12S	c.34G>A	COSM517	KRAS-M4	1%
	VIC	KRAS codon 12	p.G12R	c.34G>C	COSM518	KRAS-M5	1%
12	FAM	KRAS codon 13	p.G13D	c.38G>A	COSM532	KRAS-M7	1%

Note:

- *This panel may also detect additional mutations within the same codon in tubes 4–7.*