AmoyDx® Thyroid Cancer PCR Panel

Instructions for Use

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

REF	8.01.0261	10 tests/kit	For SLAN-96S
	8.01.0262	10 tests/kit	For QuantStudio5

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Background

Thyroid cancer is the most common malignant tumor of the endocrine system, with over 586,000 new cases reported globally in 2020 [1]. The predominant pathological subtype is differentiated thyroid carcinoma (DTC), which includes papillary thyroid carcinoma (PTC), accounting for 80%-85%, and follicular thyroid carcinoma (FTC), accounting for 10%-15% [2]. While both PTC and FTC belong to DTC, they exhibit significant differences in molecular characteristics and prognosis: PTC is primarily driven by BRAF V600E mutations (40%-70%) and RET fusions, often associated with lymph node metastasis, whereas FTC is more frequently characterized by RAS family mutations (NRAS, HRAS, KRAS) and PAX8-PPARG fusions, with a higher tendency for vascular invasion and distant metastasis [3]. Additionally, molecular alterations such as TERT promoter mutations, PIK3CA abnormalities, and NTRK fusions are enriched in poorly differentiated (PDTC) and anaplastic thyroid carcinoma (ATC), indicating tumor dedifferentiation and increased aggressiveness [4].

Current international consensus guidelines, such as the 2015 American Thyroid Association (ATA) Guidelines and the 2023 NCCN Clinical Practice Guidelines for Thyroid Carcinoma, emphasize the critical role of molecular biomarker interactions in risk stratification and personalized treatment [5-6]. For instance, co-occurrence of BRAF V600E and TERT promoter mutations significantly elevates disease-specific mortality in PTC to 30%, far exceeding that of patients with a single mutation [7]. Similarly, RAS mutations combined with PIK3CA abnormalities are strongly associated with radioiodine-refractory disease [8].

Based on these insights, the guidelines strongly recommend comprehensive multi-gene testing of preoperative fine-needle aspiration (FNA) specimens (Bethesda III/IV category) and postoperative tissue to enhance diagnostic accuracy, predict recurrence risk, and identify patients who may benefit from targeted therapies, such as TRK inhibitors for NTRK fusion-positive cases [9].

Intended Use

The AmoyDx® Thyroid Cancer PCR Panel is a qualitative real-time PCR assay designed for the detection of 21 single nucleotide variants (SNVs) across the BRAF, NRAS, HRAS, KRAS, TERT, and PIK3CA genes, as well as 12 gene fusions involving RET, PPARG, NTRK1, and NTRK3. The assay is compatible with FFPE tissue, fresh frozen tissue, and fine-needle aspiration (FNA) samples.

This kit is for research use only and must be operated by trained professionals within a controlled laboratory environment.

The AmoyDx® Thyroid Cancer PCR Panel is compatible with the SLAN-96S and QuantStudio5 platforms for testing and data collection. Results can be analyzed manually or using ARAS, an analytical tool designed to assist in the interpretation of test results.

Principles of the Procedure

The AmoyDx® Thyroid Cancer PCR Panel integrates ADx-ARMS® and Reverse Transcription PCR (RT-PCR) technologies with real-time PCR, utilizing specific primers and multi-fluorescent channel probes (FAM, VIC, ROX) to detect a broad range of DNA mutations and RNA fusions, thereby improving detection coverage and data output.

The ADx-ARMS® technology refines traditional ARMS methods through optimized primer and probe design. When the 3’ end of the primer perfectly matches the mutant template, efficient amplification occurs, generating detectable signals via FAM or ROX-labeled fluorescent probes. Conversely, if the 3’ end of the primer mismatches the wild-type template, no amplification occurs, effectively eliminating interference from wild-type DNA. This design significantly enhances the specificity and sensitivity of mutation detection.

Fusion detection is achieved through specific primers and probes designed around the fusion junctions, combined with a one-step RT-PCR approach for RNA-based fusion gene analysis. Fusion events are indicated by FAM or ROX signals, while an internal control targeting a housekeeping gene ensures RNA quality and sample integrity, monitored via the VIC channel.

To minimize false positives caused by PCR product contamination, the PCR amplification system includes UNG (Uracil-N-Glycosylase) enzyme, which selectively cleaves dU-containing PCR fragments, preventing carryover contamination and ensuring reliable results.

Kit Contents

The AmoyDx® Thyroid Cancer PCR Panel includes TMG Reaction Mix (8-tube strips), TMG Enzyme Mix A, TMG Enzyme Mix B, TMG Positive Control A, and TMG Positive Control B.

Table 1 Kit Contents

Content	Main Ingredients	Quantity
TMG Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	12 strips
TMG Enzyme Mix A	Reverse Transcriptase, Taq DNA Polymerase, UNG Enzyme	35 µL/tube ×1
TMG Enzyme Mix B	Taq DNA Polymerase, UNG Enzyme	35 µL/tube ×1
TMG Positive Control A	Cell-line RNA	60 µL/tube ×1
TMG Positive Control B	Plasmid DNA, Wild-type DNA	150 µL/tube ×1

Note:

- *Do not mix reagents from different batches.*

Each 8-tube strip is designed to test a single sample, detecting both SNVs and gene fusions.

1) Tubes 1-2 contain reagents for RNA fusion detection along with internal controls. FAM and ROX signals indicate fusion events in RET, PPARG, NTRK1, and NTRK3, while the VIC signal monitors RNA quality.
2) Tubes 3-7 contain reagents for DNA mutation detection along with internal controls. FAM and ROX signals indicate mutations in BRAF, NRAS, HRAS, KRAS, TERT, and PIK3CA, while the VIC signal monitors DNA quality.
3) Tube 8 contains reagents for DNA external control, assessing the DNA quality and overall procedure, which is indicated by FAM and ROX signal.

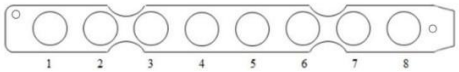
Table 2 Composition of the 8-tube Strip

Tube Number	Fluorescence	Target	Volume
1	FAM	RET	35 µL
	VIC	Internal Control	
	ROX	NTRK1	
2	FAM	PPARG	35 µL
	VIC	Internal Control	
	ROX	NTRK3	
3	FAM	NRAS	35 µL
	VIC	Internal Control	
	ROX	PIK3CA	

4	FAM	HRAS	35 μL
	VIC	Internal Control	
	ROX	PIK3CA	
5	FAM	KRAS	35 μL
	VIC	Internal Control	
	ROX	KRAS	
6	FAM	TERT Promoter	35 μL
	VIC	Internal Control	
	ROX	KRAS	
7	FAM	BRAF	35 μL
	VIC	Internal Control	
8	FAM	External Control	35 μL
	ROX	External Control	

Note:

- *Please distinguish Tube 8 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 expiration of the kit. The recommend maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

4) Compatible PCR instruments: SLAN-96S, QuantStudio5

5) DNA extraction kit: AmoyDx® FFPE DNA/RNA Kit is recommended for FFPE samples, AmoyDx® Tissue DNA Kit and AmoyDx® Tissue RNA Kit are recommended for tissue samples.

6) DNA quantification kit: Spectrophotometers, NanoDrop™ 1000/2000 is recommended.

7) Mini centrifuge with rotor for centrifuge tubes.

8) Mini centrifuge with rotor for PCR tubes.

9) Vortexer.

10) Nuclease-free centrifuge tubes.

11) Nuclease-free PCR tubes and caps.

12) Adjustable pipettors and filtered pipette tips for handling DNA.

13) Tube racks.

14) Disposable powder-free gloves.

15) Sterile, nuclease-free water.

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. Sample Requirements and Extractions

- The recommended sample types include FFPE tissue, fresh frozen tissue, or fine needle aspiration (FNA) samples from thyroid cancer patients. Samples should contain an adequate proportion of tumor cells (recommended ≥20%; for lower tumor content, enrichment is advised). Tissue samples should be extracted within 24 hours or stored at -20±5°C for no more than 6 months. FFPE samples should be stored for no more than 2 years, with a section thickness of 5–10 μm and at least 5 sections per sample.
- After DNA and RNA extraction, a spectrophotometer (e.g., Nanodrop 1000/2000) is recommended to measure concentration. The ideal concentrations are >2 ng/μL for DNA and >10 ng/μL for RNA, with an OD_{260}/OD_{280} ratio of 1.5–2.3.
- RNA should be diluted to 10–100 ng/μL with nuclease-free water, and DNA to 2 ng/μL using 1×TE buffer (pH 8.0).
- For optimal results, testing should be performed immediately after determining DNA and RNA concentrations. If storage is necessary, keep DNA at -20±5°C for up to 6 months and RNA at -20±5°C for up to 3 months.

Note:

- *The No Template Control (NTC, nuclease-free water) should be included in parallel during the nucleic acid extraction process.*

2. DNA Mix Preparation

During each PCR process, the samples must be tested alongside a Positive Control (PC) and a No Template Control (NTC, nuclease-free water) to ensure result accuracy.

1) Thaw the TMG Reaction Mix (sufficient for samples as well as PC and NTC), TMG Reaction Positive Control A and Reaction Positive Control B.
2) Vortex the above tubes to assure no frozen components exist (10-15 seconds).
3) Spin the above tubes and TMG Enzyme Mix A&B briefly to collect the contents at the bottom of the tubes (10-15 seconds).
4) Place the above tubes into an ice rack.
5) Prepare Master Mix A by pre-mixing TMG Enzyme Mix A with the samples, Positive Control A (PC), and No Template Control (NTC). The mixtures should be prepared as follows:
   a) S-Mix A: Sample RNA mix
   b) P-Mix A: Positive Control mix
   c) N-Mix A: No Template Control mix (NTC)

   If multiple RNA samples are tested, label them accordingly, such as S1-Mix A, S2-Mix A, etc., to ensure proper identification and tracking.

Table 3 Master Mix A Composition

Reagent	Volume per test
Sample (RNA, PC, NTC)	12.5 μL
TMG Enzyme Mix A	2.5 μL

6) Prepare Master Mix B by pre-mixing TMG Enzyme Mix B with the samples, Positive Control B (PC), and No Template Control (NTC). The mixtures should be prepared as follows:
   a) S-Mix B: Sample DNA mix

b) P-Mix B: Positive Control mix

c) N-Mix B: No Template Control mix (NTC)

If multiple DNA samples are tested, label them accordingly, such as S1-Mix B, S2-Mix B, etc., to ensure proper identification and tracking.

Table 4 Master Mix B Composition

Reagent	Volume per test
Sample (DNA, PC, NTC)	33 µL
TMG Enzyme Mix B	2 µL

7) Mix the Master Mix A & Master Mix B by vortexing and briefly centrifuge (10-15 seconds each).

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.*

3. Plate Setup

A single 96-well plate can accommodate up to 10 samples, one PC, and one NTC as seen in Figure 1 below.

1) Dispense 6 µL of Master Mix A into tubes 1-2 and dispense 5 µL of the exact Master Mix B into tubes 3-8.

2) Seal the 8-tube strips with the optical sealing caps.

3) Briefly centrifuge (10-15 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 8-tube strips into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Figure 1.

Tube 1	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 2	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 3	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 4	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 5	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 6	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 7	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC
Tube 8	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NTC	PC

Figure 1 Layout for a Single Test Plate Analyzing 10 Unknown Samples

4. Instrument Setup

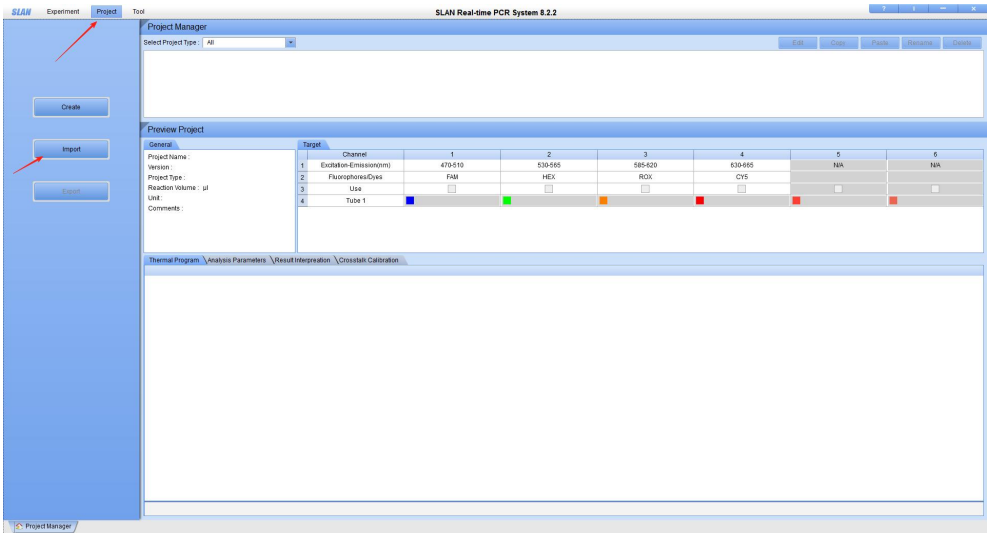
Table 5 Real-Time PCR Parameters

Stage	Cycles	Temperature	Time	Data Collection
1	1	42℃	5 min	/
		95℃	5 min	
2	10	95℃	25 s	/
		64℃	20 s	/
		72℃	20 s	/
3	36	93℃	25 s	/
		60℃	35 s	FAM, VIC, ROX
		72℃	20 s	/
4	1	40℃	30 s	/

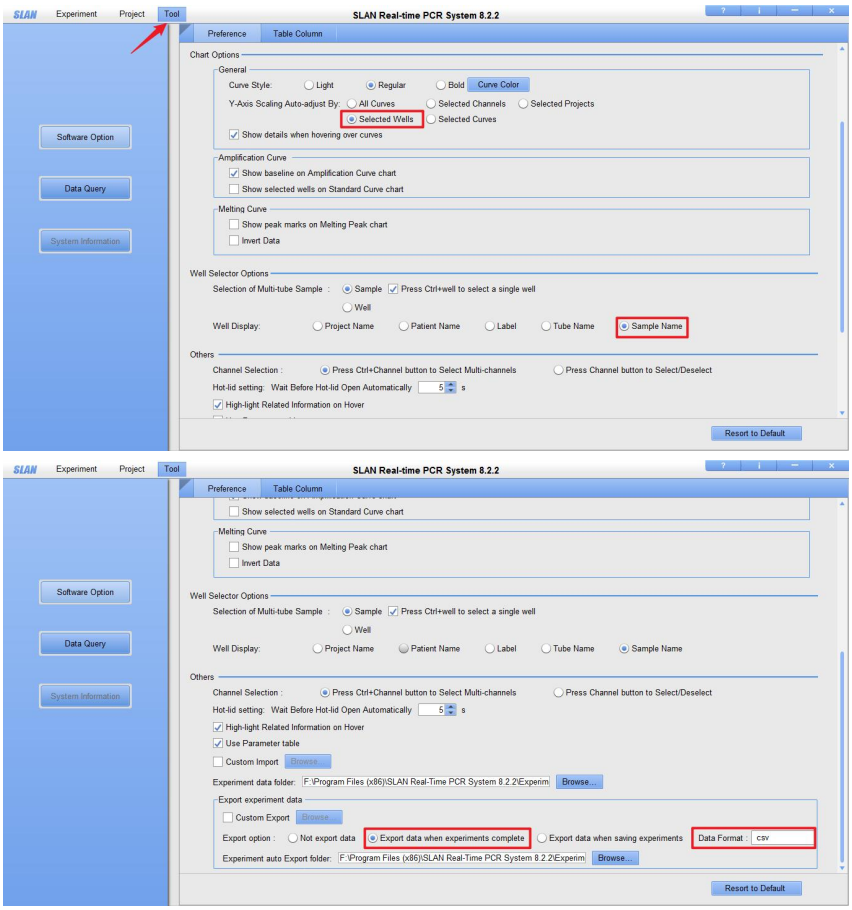
To complete the instrument setup, please follow the provided instructions to import the real-time PCR run configuration file. Ensure all parameters are correctly configured according to the file for optimal performance.

- SLAN-96S

1) Open the SLAN-96S software.
2) Click “Project” in the top bar.
3) Click “Import” in the left sidebar.
4) Select “AmoyDx Thyroid Cancer PCR Panel.prj” provided by AmoyDx. If the project file is not available, please reach out to your regional Field Application Scientist.



5) Click the “Tool” button in the top bar and set the following parameters:
   a) Select “Selected Wells” for “Y-Axis Scaling Auto-adjust By”.
   b) Select “Sample Name” for “Well Display”
   c) 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.

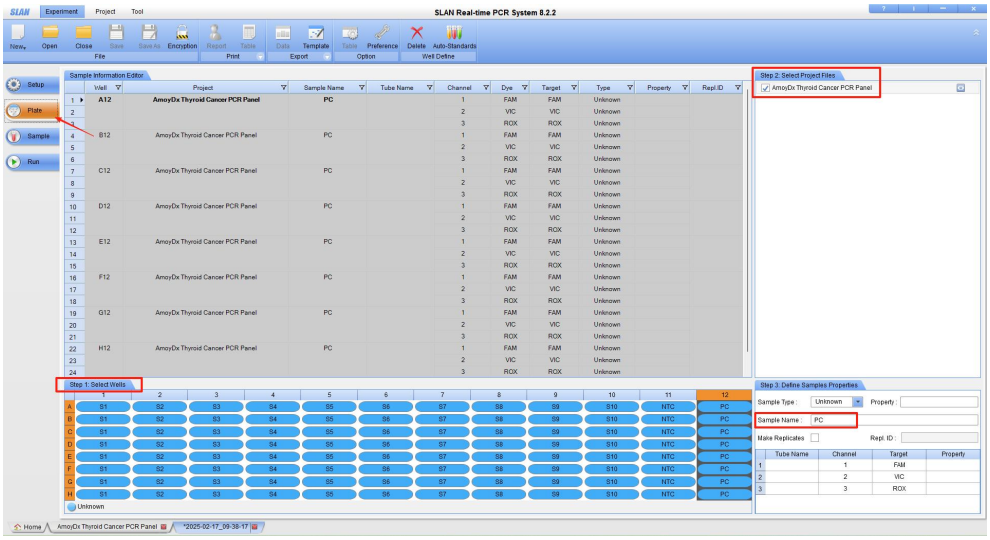
8) Set the experiment properties per your request.

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 Thyroid Cancer PCR Panel.prj”.

   c) Insert the sample names.

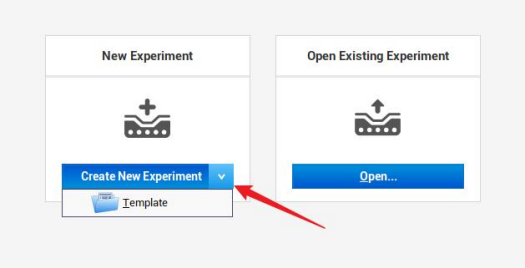


10) Click the “Run” button on the left sidebar and click “Start” to start the PCR process.

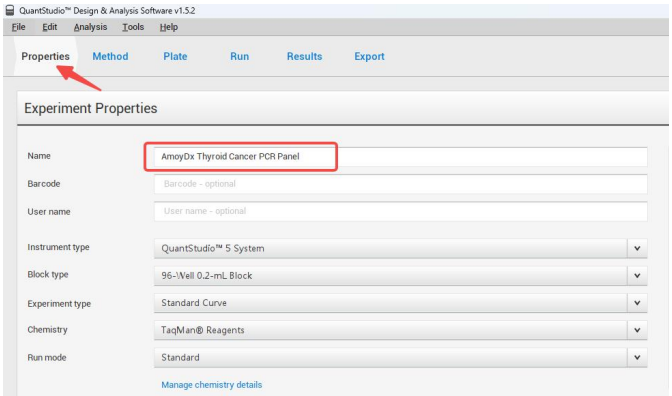
- QuantStudio5

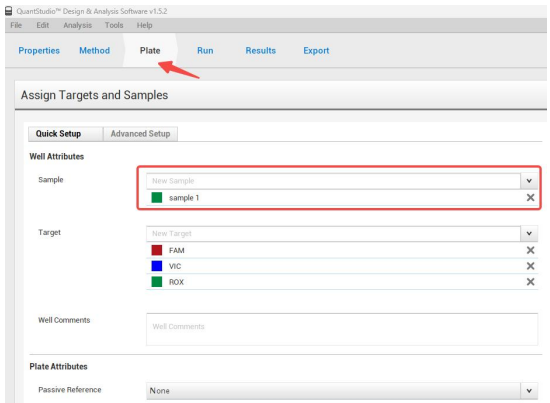
1) Open the QuantStudio5 software.

2) Click the “arrow” next to the "Create New Experiment" button to upload the template of “AmoyDx Thyroid Cancer PCR Panel.edt” provided by AmoyDx. If the template is not available, please reach out to your regional Field Application Scientist.



3) Set the experiment name and sample name per your request.





4) Leave other parameters by default.

5. Result Interpretation

Threshold Setting: For analysis, select a single reaction tube and corresponding fluorescence signal sequentially by tube number (Tubes 1-8), along with the respective Positive Control (PC) and No Template Control (NTC) tubes. Adjust the threshold to 5% of the PC’s peak fluorescence at the 36th cycle to determine the Ct values for each reaction tube.

No Template Control and Positive Control Analysis

1) **NTC (No Template Control):** The FAM signals in tubes 1-8, VIC signals in tubes 1-7 and ROX signals in tubes 1-6 & 8 for the NTC normally should exhibit no amplification curves.
   a) If any FAM signals in tubes 1-7 and ROX signals in tubes 1-6 show amplification, the experiment is deemed invalid and should be repeated, as this suggests potential contamination.
   b) If the VIC signals in tube 1-7, FAM and ROX signals in tube 8 show occasional amplification while the FAM signals in tubes 1-7, ROX signals in tubes 1-6 do not, this does not impact the validity of the mutation detection results, and analysis may proceed as planned.
2) **PC (Positive Control):** The FAM signals in tubes 1-8, VIC signals in tubes 1-7 and ROX signals in tubes 1-6 & 8 of the positive control should all demonstrate clear amplification. The Ct values for these signals should be below 25.

RNA Fusion Analysis

1) **Internal Control (IC):** For tubes 1-2 of the sample, the VIC signal should show clear amplification curves with Ct values <36, and at least one of the two tubes must have a Ct value ≤25.
   a) If both VIC Ct values are <36 and at least one is ≤25: Proceed with the analysis.
   b) If either VIC Ct value is ≥36 or both are >25: This suggests potential issues such as insufficient RNA loading or poor RNA quality. Re-test or re-extract the RNA as necessary.
2) **RNA Fusion Analysis:** Set the threshold based on the fluorescence value of the positive control (PC) at the final cycle to accurately determine the Ct values for each fusion detection tube. Check the FAM, ROX signal amplifications in tubes 1-2. Classify the sample results as negative or positive in accordance with the detailed criteria specified in Table 6.
   a) If the Ct values for FAM, ROX fall within the negative Ct range, the sample is considered negative in that tube.
   b) If the Ct values for FAM, ROX fall within the positive range, the sample is considered positive in that tube.

Table 6 RNA Fusion Positive and Negative Ct Range

	Tube 1 FAM RET	Tube 1 ROX NTRK1	Tube 2 FAM PPARG	Tube 2 ROX NTRK3
Positive	Ct<28	Ct<30	Ct<28	Ct<30
Negative	Ct≥28	Ct≥30	Ct≥28	Ct≥30

DNA Mutation Analysis:

1) **External Control (EC):** The FAM and ROX signals in tube 8 of the sample should display clear amplification curves with Ct values between ≥17 and ≤25.
   a) If the FAM & ROX signal Ct value in tube 8 is >25 or if no significant amplification curve is observed, this suggests the presence of PCR inhibitors, insufficient DNA concentration, or severe nucleic acid degradation. In such cases, DNA extraction should be repeated, and the experiment should be conducted anew. However, if positive amplification is observed and the result is determined to be positive of FAM signals in tubes 3-7 or ROX signals in tubes 3-6, the result should still be considered valid.
   b) If the FAM & ROX signal Ct value in tube 8 is <17, this indicates an overloaded DNA input. The DNA concentration should be diluted, and the test should be repeated. However, if the overloaded results show no amplification or fall within the negative range of FAM signals in tubes 3-7 and ROX signals in tubes 3-6, the negative result remains valid.
2) **Internal Control (IC):** The internal control VIC signal in tubes 3-7 of sample should display a clear amplification curve with a Ct <36.
   a) If all VIC Ct values are <36, proceed with the analysis.
   b) If any VIC Ct values are ≥36, this indicates potential issues such as missing DNA loading or poor DNA quality. Re-test or re extract the DNA as needed.
3) **DNA Mutation Analysis:** Set the threshold based on the fluorescence value of the positive control (PC) at the final cycle to accurately determine the Ct values for each mutation detection tube. Check the FAM signal amplifications in tubes 3-7, ROX signal

amplifications in tubes 3 to 6. Classify the sample results as negative or positive in accordance with the detailed criteria specified in Table 7.

a) If the Ct values for FAM, ROX fall within the negative Ct range, the sample is considered negative in that tube.

b) If the Ct values for FAM, ROX fall within the positive A range, the sample is considered positive in that tube.

c) If the Ct values for FAM, ROX fall within the positive B range, calculate the ΔCt value:

ΔCt Value=Mutant FAM, ROX Ct value − External Control FAM, ROX Ct value

If the ΔCt value is less than the ΔCt Cut-off value, the sample is considered positive in that tube. Otherwise, it is considered negative.

Table 7 DNA Mutation Positive and Negative Ct Range

		Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
FAM	Positive A	Ct<29	Ct<30	Ct<30	Ct<28	Ct<31
	Positive B	29≤Ct<31	30≤Ct<32	30≤Ct<32	28≤Ct<30	31≤Ct<33
	ΔCt Cut-off	9	9	9	9	10
	Negative	Ct≥31	Ct≥32	Ct≥32	Ct≥30	Ct≥33
ROX	Positive A	Ct<31	Ct<30	Ct<29	Ct<31	NA
	Positive B	31≤Ct<33	30≤Ct<32	29≤Ct<31	31≤Ct<33	
	ΔCt Cut-off	9	9	9	9	
	Negative	Ct≥33	Ct≥32	Ct≥31	Ct≥33	

4) **Cross Reactivity:** KRAS positive samples with mutations in tube 5 ROX may cause cross-signals in tube 6 ROX. When a sample shows dual-positive results in tube 5 & 6 (as interpreted according to Table 7), if the Ct value in tube 5 ROX is smaller, the positive result in tube 5 ROX is determined as the true positive. Then, calculate ΔCt value for tube 6 ROX and use the cross-signal cut-off values listed in Table 8 to determine whether tube 6 ROX is displaying cross-signals. If the Ct value in tube 6 ROX is smaller, the sample could be interpreted as dual-positive.

a) If ΔCt is less than the cross-signal cut-off value, it is determined as true positive in the tube.

b) Otherwise, it is determined as negative in the tube by cross-signals.

Table 8 Cross-signal cut-off values

Reaction Tubes / True Positive	Tube 6 ROX
Tube 5 ROX	5.5

5) Automated Analysis:

ARAS is a proprietary software installed on a stand-alone workstation PC developed by AmoyDx intended to be used as a tool to aid in the interpretation of AmoyDx® Thyroid Cancer PCR Panel. Upon the PCR run completion, import the PCR data into the ARAS, to determine sample's mutation status based on the Ct values of the target mutations.

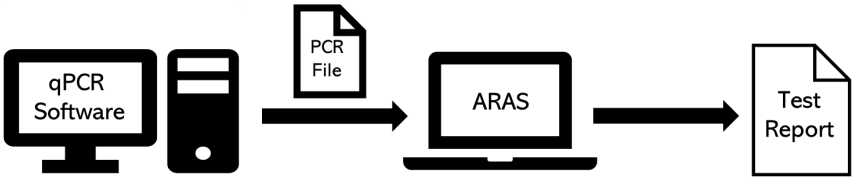


Figure 2 PCR Data to ARAS Workflow Overview

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 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) Following the instructions, testing was performed on positive corporate reference material. The concordance rate for positive reference materials was 100%.
3) Following the instructions, testing was performed on negative corporate reference materials, and the concordance rate for negative reference materials was 100%.
4) Under the specified testing conditions, the kit's limit of detection for the covered DNA mutation types ranges from 1% to 5% at 10 ng DNA input and 125 copies per reaction for cell-line RNA fusions (see Appendix for details).
5) Precision testing was conducted on 15 precision reference samples across inter-day, intra-day, inter-operator, and inter-batch conditions. The negative precision reference samples yielded negative results, while the positive precision reference samples tested positive for the corresponding mutation detection system, with the coefficient of variation (CV, %) for Ct values being less than 5%.

Note:

- *The above performance characteristics are based on the SLAN-96S platform. Performance may vary slightly when using other types of instruments.*

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 entirely rule out the presence of mutations or fusion genes, as factors such as insufficient tumor cells in the sample, excessive degradation, or DNA mutation/fusion RNA concentrations below the detection limit of the panel may also lead to a negative outcome.
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) Tumor tissue (or cells) may exhibit significant heterogeneity, and sampling from different regions could yield varying test results.
6) The detection scope of this panel is limited to the known targets specified in the appendix and does not include the detection of gene mutation types outside the kit's covered range.
7) The test results obtained with this kit are for research purposes only and should not be used for diagnostic procedures.

Reference

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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 1 Fusion

Tube	Fluorescence	Target	Fusion Type
1	FAM	*RET*	*CCDC6* exon1；*RET* exon12
			CCDC6 exon8；*RET* exon12
			NCOA4(*RFG*) exon8；*RET* exon12
	ROX	*NTRK1*	*TPM3* exon7；*NTRK1* exon10
			IRF2BP2 exon1；*NTRK1* exon10
			SQSTM1 exon5；*NTRK1* exon10
			TPR exon21；*NTRK1* exon10
2	FAM	*PPARG*	*PAX8* exon10；*PPARG* exon2
	ROX	*NTRK3*	*ETV6* exon4；*NTRK3* exon14
			EML4 exon2；*NTRK3* exon14
			SQSTM1 exon5；*NTRK3* exon14
			RBPMS exon5；*NTRK3* exon14

Appendix 1 Mutation

Tube	Fluorescence	Target	MUTATION_AA	MUTATION_CDS	COSMIC ID	Name	LoD
3	FAM	*NRAS*	p.Q61R	c.182A>G	COSM584	NRAS-M1	1%
			p.Q61K	c.181C>A	COSM580	NRAS-M2	2%
			p.Q61L	c.182A>T	COSM583	NRAS-M5	1%
			p.Q61H	c.183A>C	COSM586	NRAS-M8	1%
	ROX	*PIK3CA*	p.E545K	c.1633G>A	COSM763	PI-M4	2%
4	FAM	*HRAS*	p.G12V	c.35G>T	COSM483	HRAS-M2	2%
			p.G13R	c.37G>C	COSM486	HRAS-M4	3%
			p.Q61R	c.182A>G	COSM499	HRAS-M5	2%
			p.Q61K	c.181C>A	COSM496	HRAS-M6	1%
	ROX	*PIK3CA*	p.H1047R	c.3140A>G	COSM775	PI-M1	2%
5	FAM	*KRAS*	p.Q61R	c.182A>G	COSM552	KRAS-M16	1%
	ROX		p.G12D	c.35G>A	COSM521	KRAS-M1	5%
			p.G12A	c.35G>C	COSM522	KRAS-M2	2%
			p.G12V	c.35G>T	COSM520	KRAS-M3	1%
			p.G12S	c.34G>A	COSM517	KRAS-M4	5%
			p.G12R	c.34G>C	COSM518	KRAS-M5	1%
			p.G13C	c.37G>T	COSM527	KRAS-M14	1%
6	FAM	*TERT* promoter	p.C228T	c.1-124C>T	COSM1716558	TERT-M1	1%
			p.C250T	c.1-146C>T	COSM1716559	TERT-M2	1%
	ROX	*KRAS*	p.G12C	c.34G>T	COSM516	KRAS-M6	3%
7	FAM	*BRAF*	p.V600E	c.1799T>A	COSM476	BRAF-M1	1%

Note:

- *The above performance characteristics are based on the SLAN-96S platform. Performance may vary slightly when using other types of instruments.*