AmoyDx® Super-ARMS ESR1 PCR Kit

Instructions for Use

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

REF	8.01.0306	14 tests/kit	For QuantStudio 5
	8.01.0307	14 tests/kit	For SLAN-96S

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Version: P1.0

Sept 2024

Background

For patients with hormone receptor (HR)-positive advanced breast cancer, resistance to endocrine therapy (ET) represents a critical turning point. Although most HR-positive breast cancers initially respond to first-line ET, resistance eventually develops. Consequently, second-line ET monotherapy typically offers a median progression-free survival (PFS) of only 2–6 months, compared to 1–4 years with first-line treatment [1]. A key mechanism underlying this endocrine resistance is mutation in the ligand-binding domain (LBD) of Estrogen Receptor 1 (ESR1), which encodes estrogen receptor α (ER). Over the past decade, these mutations have been the subject of extensive research, focusing on their biochemical and molecular effects, implications for treatment selection, and potential therapeutic targets [2]. All ESR1 resistance mutations occur within the LBD, with the most common being D538G and Y537S. Other significant mutations include Y537N, Y537C, L536H, L536P, L536R, S463P, and E380Q [3-7].

These may cause changes in protein conformation, leading to ligand-independent activation of the ER signaling pathway, which can ultimately result in endocrine therapy resistance and promote distant metastasis of tumors. In primary breast cancer, the mutation frequency of the ESR1 gene is less than 5%; however, in advanced breast cancer patients, particularly those who have previously received Aromatase Inhibitor (AI) therapy, the ESR1 mutation frequency significantly increases to 14% to 54% [8].

On January 27, 2023, the FDA approved ORSERDU® (elacestrant), a targeted therapy for HR+/HER2- advanced or metastatic breast cancer with ESR1 mutations, based on the results of the EMERALD trial. On September 20, 2023, the European Commission approved ORSERDU® (elacestrant) as a monotherapy for postmenopausal women and men with estrogen receptor (ER)-positive, HER2-negative, locally advanced or metastatic breast cancer (mBC) with an activating ESR1 mutation. This approval applies to patients whose disease has progressed after at least one line of endocrine therapy.

Intended Use

The AmoyDx® Super-ARMS ESR1 PCR Kit is a qualitative real-time PCR assay developed for the detection of 29 somatic mutations in the ESR1 gene across exons 4, 5, 6, 7, and 8, using circulating free DNA (cfDNA) extracted from plasma samples. cfDNA, originating from apoptotic and necrotic tumor cells, can be detected in the peripheral blood of breast cancer patients. Plasma-based detection of ESR1 mutations offers a non-invasive method to assess the mutation status in breast cancer patients. However, due to the highly fragmented nature and low concentration of cfDNA in the bloodstream, especially in early-stage breast cancer patients compared to those with advanced disease, peripheral blood testing is more suitable for late-stage cases. This kit is intended for research use only and must be operated by trained professionals in a controlled laboratory environment.

This kit is compatible with the QuantStudio 5 and SLAN-96S platforms for testing and data collection. Results could be analyzed manually or using ARAS, a tool to aid in the interpretation of AmoyDx® Super-ARMS ESR1 PCR Kit.

Principles of the Procedure

The AmoyDx® Super-ARMS ESR1 PCR Kit integrates advanced Super-ARMS® technology with real-time PCR, utilizing specific primers and multi-fluorescent channel probes (FAM, VIC, ROX, CY5) to detect a range of ESR1 mutations in cfDNA from plasma samples. This

approach significantly enhances detection coverage and information yield.

The Super-ARMS® technology improves upon traditional ARMS methods by refining primer and probe design. When the 3' end of the primer perfectly matches the mutant template, efficient amplification occurs. The resultant products are detected using fluorescent probes labeled with FAM, ROX, and CY5. In contrast, if the 3' end of the primer does not match the wild-type template, amplification does not occur, thus avoiding interference from wild-type DNA. This advanced design markedly improves both the specificity and sensitivity of mutation detection.

The AmoyDx® Super-ARMS ESR1 PCR Kit includes **ESR1 Reaction Mix** (12-tube strips), **ESR1 Reaction Mix A**, **ESR1 Enzyme Mix**, and **ESR1 Positive Control**. The kit is designed with 12-tube strips, each strip capable of testing 2 samples. Tubes 2-6 of each strip contain reagents for detecting 29 ESR1 gene mutations along with internal control reagents. Mutation signals are indicated by FAM/ROX/CY5, while the internal control is indicated by VIC. The internal control targets a relatively conserved region of the human ESR1 gene, used to monitor the cfDNA quality of plasma samples and the PCR process. The internal control amplifies in all samples, regardless of the presence of ESR1 gene mutations. Tube 1 contain external control reagents for the ESR1 gene, with signals also indicated by FAM/ROX/CY5. The external control serves as part of the result interpretation and monitors the cfDNA quality from plasma samples and the accuracy of the results, targeting a conserved region of the ESR1 gene. 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.

Table 1 ESR1 Mutations Detected by the Kit

Exon	Number of ESR1 Mutations	Amplification Target Sequence Length
4	3	80–120 bp
5	4	80–110 bp
6	3	70–110 bp
7	2	95–120 bp
8	17	85–160 bp

Kit Contents

This kit contains the following materials sufficient for up to 14 patient specimens per kit:

Table 2 Kit Contents

Content	Main Ingredients	Quantity
ESR1 Reaction Mix	Primers, Probes, dNTPs	8 strips
ESR1 Reaction Mix A	H_2O, Buffer, Mg^{2+}	130 µL/tube ×16
ESR1 Enzyme Mix	Taq DNA Polymerase, UNG Enzyme	50 µL ×1
ESR1 Positive Control	Plasmid DNA, Wild-type DNA	300 µL /tube ×1

Note:

- *Do not mix reagents from different batches.*
- *The ESR1 Positive Control contains a mixture of synthetic plasmid DNA sequences that correspond to a representative mutation per*

reaction detected by this kit in a background of wild-type DNA.

Table 3 Composition of the 12-tube Strip

Tube Number	Fluorescence	Target	Volume
1	FAM, ROX, CY5	External Control	10 μL
2	FAM	p.D538G/p.Y537S/p.Y537N/p.L536P	10 μL
	VIC	Internal Control	
	ROX	p.E380Q	
3	FAM	p.Y537C/p.L536R	10 μL
	VIC	Internal Control	
	ROX	p.S463P	
4	FAM	p.L536H/p.H524L/p.L549P/p.Y537H	10 μL
	VIC	Internal Control	
	ROX	p.G442R	
	CY5	p.L370F/p.E380K	
5	FAM	p.L536Q/p.V534E/p.Y537D/p.D538Y/p.L536K	10 μL
	VIC	Internal Control	
	ROX	p.L469V	
	CY5	p.V422del	
6	FAM	p.D538N/p.L536G	10 μL
	VIC	Internal Control	
	ROX	p.L379I/p.V418E	
	CY5	p.G344D/p.H356D/p.H356Y	

Note:

- *Please distinguish Tube 6 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

1) Compatible PCR instruments: QuantStudio 5, SLAN-96S

2) Blood collection tube: AmoyDx® Cell-free DNA Protection Vacuum Tube is recommended.

3) DNA extraction kit: AmoyDx® Circulating DNA Kit is recommended.

4) DNA quantification kit: QuantiFluor dsDNA System (Promega) is recommended.

5) Mini centrifuge with rotor for centrifuge tubes.

6) Mini centrifuge with rotor for PCR tubes.

7) Vortexer.

8) Nuclease-free centrifuge tubes.

9) Nuclease-free PCR tubes and caps.

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

11) Tube racks.

12) Disposable powder-free gloves.

13) 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 DNA Extraction

- The sample type is peripheral blood from breast cancer patients, and the collected volume should not be less than 10 mL.
- It is recommended to use EDTA vacuum blood collection tubes (AmoyDx® Cell-free DNA Protection Vacuum Tube, special for cfDNA protection). Please DO NOT use heparin as an anticoagulant.
- Plasma separation is advised within 2 hours after blood collection and should not exceed 4 hours. The plasma volume should not be less than 2 mL. For plasma separation, first centrifuge at 2,000×g for 10 minutes, collect the supernatant; then centrifuge at 8,000×g for 10 minutes, and collect the supernatant again.
- If plasma separation cannot be done promptly, the vacuum blood collection tubes provided by AmoyDx can be stored at room temperature for up to one week. Separated plasma can be stored at -20±5℃ for up to 2 years.
- The AmoyDx® Circulating DNA Kit has been validated for isolating DNA from plasma samples specifically for ESR1 mutation testing, ensuring reliable assay performance. After extracting cfDNA, it is recommended to use a nucleic acid quantification kit (such as Quantus or Qubit, using fluorescent dye) to measure the cfDNA concentration. The ideal cfDNA concentration should range from 0.1 to 3 ng/μL. For concentrations ≥3 ng/μL, it is recommended to dilute it to 2 ng/μL using TE (pH 8.0) or nuclease-free water before testing. If the cfDNA concentration is <0.1 ng/μL, re-extraction is advised to ensure the accuracy of the test results.
- After determining the concentration of extracted cfDNA, it is recommended to proceed with testing immediately; otherwise, store the extracted DNA at below -20±5℃, and do not store it for more than 3 months.

The kit evaluates DNA input amount using External Controls (FAM) Ct to confirm sufficient loading. Since the DNA extracted from plasma samples may vary due to fragmentation, sample load adjustments may be needed.

Note:

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

2. DNA 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 **ESR1 PCR Strips** (sufficient for samples as well as PC and NTC), **ESR1 Reaction Mix A**, **ESR1 Positive Control**.
2) Vortex the above tubes to assure no frozen components exist (10-15 seconds).
3) Spin the tubes briefly to collect the contents at the bottom of the tubes (10-15 seconds).

4) Place the above tubes into an ice rack.

5) ESR1 Reaction Mix A should be pre-mixed with ESR1 Enzyme Mix and samples (as well as control samples of PC and NTC) as a **Master Mix**. The ratio is shown as follows.

Table 4 ESR1 Master Mix Composition

Reagent	Volume per test
ESR1 Reaction Mix A	130 µL
Sample (DNA, PC, NTC)	65 µL
ESR1 Enzyme Mix	2.6 µL

6) Mix the **Master Mix** by vortexing and briefly centrifuge (10-15 seconds each).

3. Plate Setup

Six reactions (Tubes 1-6) are setup for each sample (One 12-tube strip is sufficient for 2 samples). A single 96-well plate can accommodate up to 14 samples, one PC, and one NTC as seen in Figure 2 below.

1) Dispense 30.4µL of the Master Mix into the tubes of 12-tube strips (Tubes 1-6 as one sample).

2) Seal the 12-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 in the bottom of the tubes.

4) Place the 12-tube strips into the appropriate positions of the real-time PCR instrument. A recommended plate layout is shown in Figure 1.

Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
S1	S1	S1	S1	S1	S1	S2	S2	S2	S2	S2	S2
S3	S3	S3	S3	S3	S3	S4	S4	S4	S4	S4	S4
S5	S5	S5	S5	S5	S5	S6	S6	S6	S6	S6	S6
S7	S7	S7	S7	S7	S7	S8	S8	S8	S8	S8	S8
S9	S9	S9	S9	S9	S9	S10	S10	S10	S10	S10	S10
S11	S11	S11	S11	S11	S11	S12	S12	S12	S12	S12	S12
S13	S13	S13	S13	S13	S13	S14	S14	S14	S14	S14	S14
PC	PC	PC	PC	PC	PC	NTC	NTC	NTC	NTC	NTC	NTC

Figure 1 Super-ARMS ESR1 Layout for a Single Test Plate Analyzing 14 Unknown Samples

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 Real-Time PCR Parameters

Stage	Cycles	Temperature	Time	Data Collection
1	1	95℃	10 min	/
2	15	95℃	25 s	/
		62℃	20 s	/
		72℃	20 s	/
3	30	93℃	25 s	/
		58℃	35 s	FAM, VIC, ROX, CY5
		72℃	20 s	/
4	1	40℃	10 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.

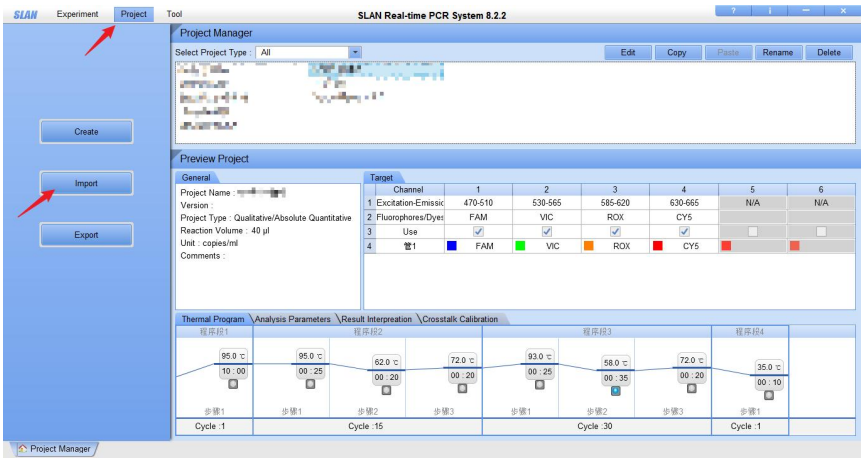
- SLAN-96S

1) Open the SLAN-96S software.

2) Click “Project” in the top bar.

3) Click “Import” in the left sidebar.

4) Select “AmoyDx Super-ARMS ESR1.prj” provided by AmoyDx. If the project file is not available, please reach out to your regional Field Application Scientist.

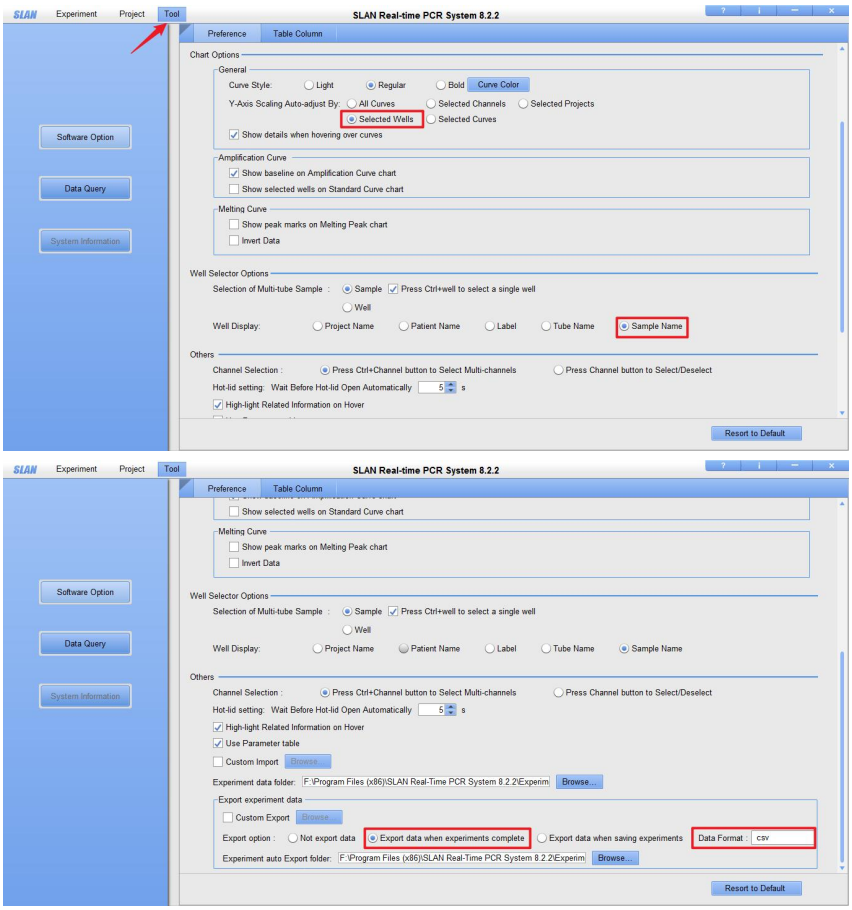


5) Click the “Tool” button in the top bar and set the following parameters:

   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.

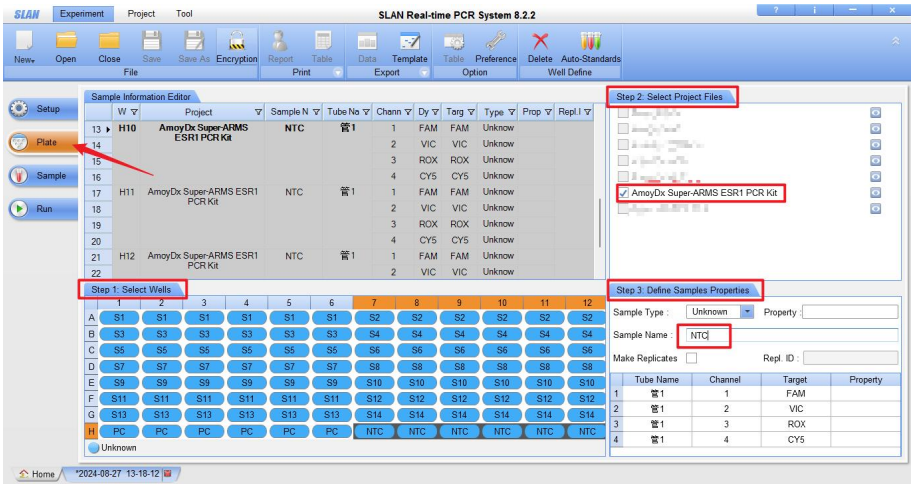
8) Set the experiment properties per your request.

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

   a) Highlight the wells you assigned the ESR1 PCR Strips.

   b) Select the project file “AmoyDx Super-ARMS ESR1.prj”.

   c) Insert the sample names.

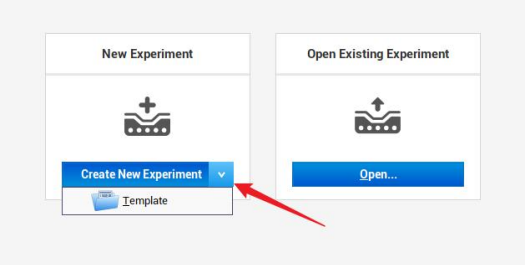


10) Click the “Run” button on the left sidebar and click “Start” to start the PCR process.

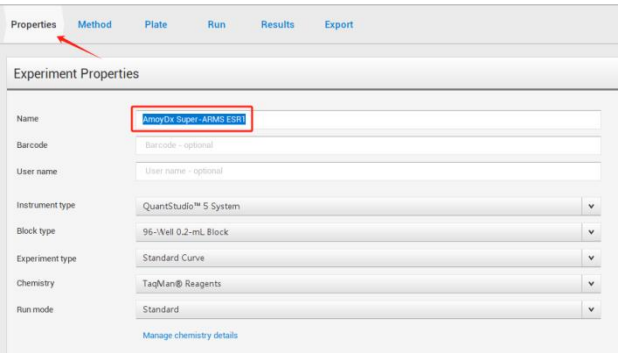
- QuantStudio 5

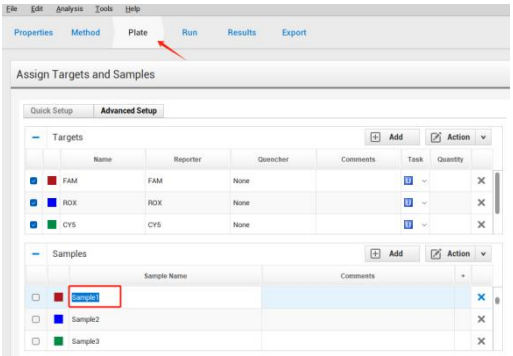
1) Open the QuantStudio 5 software.

2) Click the “arrow” next to the "Create New Experiment" button to upload the template of “AmoyDx Super-ARMS ESR1 PCR Kit.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) Click “START RUN” button to start the PCR process.

5. Result Interpretation

Threshold Setting: For analysis, select a single reaction tube and corresponding fluorescence signal sequentially by tube number (Tubes 1-6), 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 30th cycle to determine the Ct values for each reaction tube.

1) **NTC (No Template Control):** The FAM, ROX, and CY5 signals for the NTC should exhibit no amplification curves.
   a) If any of these signals in tubes 2 to 6 show amplification, the experiment is deemed invalid and should be repeated, as this suggests potential contamination.
   b) If the FAM, ROX, and CY5 signals in tube 1 and VIC signals in tubes 2 to 6 show occasional amplification while the FAM, ROX signals in tubes 2 to 6 and CY5 signals in tubes 4 to 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, ROX in tubes 1 to 6, CY5 in tube1 and tubes 4 to 6 and the VIC signals in tubes 2 to 6 of the positive control should all demonstrate clear amplification. The Ct values for these signals should be below 20.
3) **External Control (EC):** The external control FAM signal in tube 1 of the sample should display a clear amplification curve with a Ct value between ≥11 and <18.
   a) If the FAM signal Ct value is ≥18 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 in tubes 2 to 6, the result should still be considered valid.

b) If the FAM signal Ct value is <11, this indicates an overloaded of 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 in tubes 2 to 6, the negative result remains valid.

4) **Internal Control (IC):** The internal control VIC signal in tubes 2 to 6 of sample should display a clear amplification curve with a Ct <30.

a) If all VIC Ct values are <30, proceed with the analysis.

b) If any VIC Ct values are ≥30, this indicates potential issues such as missing DNA loading or poor DNA quality. Re-test or re extract the DNA as needed.

5) **ESR1 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, ROX signal amplifications in tubes 2 to 6 and the CY5 signal in tubes 4 to 6. 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, or CY5 fall within the negative Ct range, the sample is considered negative in that tube.

b) If the Ct values for FAM, ROX, or CY5 fall within the positive A range, the sample is considered positive in that tube.

c) If the Ct values for FAM, ROX, or CY5 fall within the positive B range, calculate the ΔCt value:

ΔCt Value=Mutant FAM, ROX, or CY5 Ct value − External Control FAM, ROX, or CY5 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 6 Positive and Negative Ct Range

		Tube 2	Tube 3	Tube 4	Tube 5	Tube 6
FAM	Positive A	Ct<25	Ct<24	Ct<25	Ct<25	Ct<25
	Positive B	25≤Ct <28	24≤Ct <27	25≤Ct <28	25≤Ct <27	25≤Ct <28
	ΔCt Cut-off	9	9	10	9	9
	Negative	Ct≥28	Ct≥27	Ct≥28	Ct≥27	Ct≥28
ROX	Positive A	Ct<25	Ct<25	Ct<25	Ct<26	Ct<26
	Positive B	25≤Ct <28	25≤Ct <27	25≤Ct <27	26≤Ct <28	26≤Ct <28
	ΔCt Cut-off	9	9	9	9	9
	Negative	Ct≥28	Ct≥27	Ct≥27	Ct≥28	Ct≥28
CY5	Positive A	/	/	Ct<24	Ct<24	Ct<25
	Positive B	/	/	24≤Ct <26	24≤Ct <26	25≤Ct <27
	ΔCt Cut-off	/	/	9	10	9
	Negative	/	/	Ct≥26	Ct≥26	Ct≥27

6) **Cross Reactivity:** Positive samples with certain mutations may cause cross-signals between individual mutation reaction tubes. When a sample shows positive results in 2 or more reaction tubes (as interpreted according to Table 6), first determine the reaction tube the smaller Ct value as the true positive. Then, calculate ΔCt value for the other mutation reaction tubes and use the cross-signal cut-off values listed in Table 7 to determine whether the other reaction tubes are displaying cross-signals.

   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 7 Cross-signal cut-off values

Reaction Tubes / True Positive	Tube 2 FAM	Tube 4 FAM
Tube 3 FAM		9.10
Tube 5 FAM	5.31	10.25

7) 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® Super-ARMS ESR1 PCR Kit. 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.

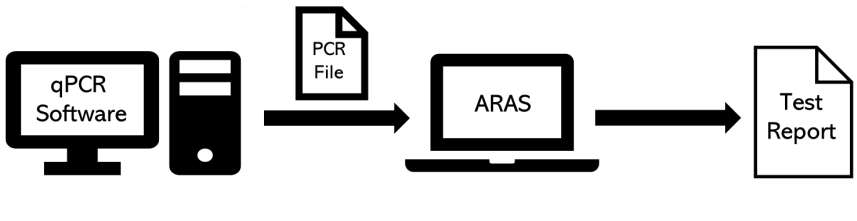


Figure 2 ESR1 PCR Data to ARAS Workflow Overview

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.

   b) PCR file from QuantStudio 5 should be in eds 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 strong, medium, and weak positive corporate reference materials containing 29 ESR1 gene mutations. The concordance rate for positive reference materials was 100%. The positive reference materials consisted of clinical positive plasma samples and DNA samples from cell lines, with DNA mutation content determined by NGS or digital PCR quantification.
3) Following the instructions, testing was performed on negative corporate reference materials, and the concordance rate for negative reference materials was 100%. The negative reference materials consisted of clinical negative plasma samples.
4) Testing of samples from common related infectious microorganisms yielded negative results. There was no cross-reactivity between sequences with similar or homologous nucleic acid sequences that could cause cross-reactions, and no false positives were detected with wild-type ESR1 DNA.
5) Under the specified testing conditions, the kit's limit of detection for the covered mutation types ranges from 0.2% to 2% at a DNA input of 5 ng (see Appendix for details).
6) The same negative precision reference material was tested 10 times, with all results negative. The same positive precision reference material was tested 10 times, with all tests detecting positive results.

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 completely exclude the presence of an ESR1 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 detection reagent is not suitable for detecting ESR1 copy number expression levels.
6) This kit is only applicable for mutation detection of plasma sample DNA and is not suitable for FFPE sample DNA detection.
7) The ESR1 gene mutation detection range of this reagent only includes the known gene mutation loci explicitly covered by the

detection reagent and does not include gene mutation loci outside the declared range of the kit.

8) The test results obtained with this kit are for research purposes only and should not be used for diagnostic procedures.

Reference

[1] Nagaraj G, Ma CX. Clinical challenges in the management of hormonereceptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer: a literature review. Adv Ther. 2021;38:109–36.

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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

Tube	Fluorescence	MUTATION_AA	Exon	MUTATION_CDS	COSMIC ID	Name	LoD
Tube 2	FAM	p.D538G	Exon 8	c.1613A>G	COSM94250	ES-M1	0.2%
		p.Y537S		c.1610A>C	COSM1074639	ES-M2	0.2%
		p.Y537N		c.1609T>A	COSM1074635	ES-M4	0.2%
		p.L536P		c.1607T>C	COSM6906109	ES-M6	0.2%
	ROX	p.E380Q	Exon 5	c.1138G>C	COSM3829320	ES-M9	0.2%
Tube 3	FAM	p.Y537C	Exon 8	c.1610A>G	COSM1074637	ES-M3	0.5%
		p.L536R		c.1607T>G	COSM4774826	ES-M7	0.5%
	ROX	p.S463P	Exon 7	c.1387T>C	COSM4771561	ES-M8	0.5%
Tube 4	FAM	p.L536H	Exon 8	c.1607T>A	COSM6843697	ES-M5	0.6%
		p.H524L		c.1571A>T	COSM6579522	ES-M15	0.5%
		p.L549P		c.1646T>C	COSM6906110	ES-M20	0.4%
		p.Y537H		c.1609T>C	COSM7449624	ES-M40	0.4%
	ROX	p.G442R	Exon 6	c.1324G>C	COSM6942951	ES-M24	0.4%
	CY5	p.L370F	Exon 5	c.1110G>C	COSM6563083	ES-M21	0.5%
		p.E380K		c.1138G>A	/	ES-M38	0.4%
Tube 5	FAM	p.L536Q	Exon 8	c.1607_1608delinsAG	COSM4766050	ES-M10	0.4%
		p.V534E		c.1601T>A	COSM4774827	ES-M11	0.3%
		p.Y537D		c.1609T>G	COSM6918757	ES-M13	0.4%
		p.D538Y		c.1612G>T	/	ES-M32	0.5%
		p.L536K		c.1606_1608delinsAAG	/	ES-M42	1.0%
	ROX	p.L469V	Exon 7	c.1405C>G	/	ES-M26	0.5%
	CY5	p.V422del	Exon 6	c.1265_1267del	COSM1074628	ES-M16	0.2%
Tube 6	FAM	p.D538N	Exon 8	c.1612G>A	/	ES-M29	1.5%
		p.L536G		c.1606_1607delinsGG	/	ES-M41	0.4%
	ROX	p.V418E	Exon 6	c.1253T>A	COSM6926544	ES-M17	0.4%
		p.L379I	Exon 5	c.1135C>A	/	ES-M37	0.6%
	CY5	p.G344D	Exon 4	c.1031G>A	COSM6921388	ES-M19	1.5%
		p.H356D		c.1066C>G	/	ES-M34	1.0%
		p.H356Y		c.1066C>T	COSM10523954	ES-M35	2.0%

Note:

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