AmoyDx® Super-ARMS ESR1 Performance Evaluation

Introduction

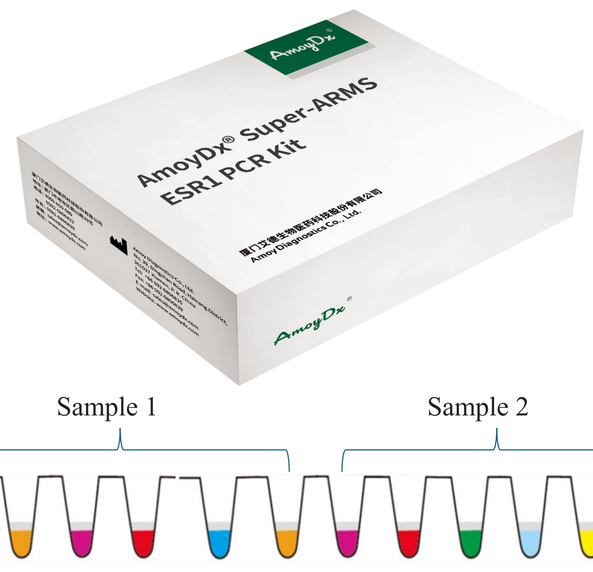
For patients with hormone receptor (HR)-positive advanced breast cancer, resistance to endocrine therapy (ET) represents a critical turning point. Although most HR-positive breast cancers initially respond to first-line ET, resistance eventually develops. Consequently, second-line ET monotherapy typically 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]. Almost 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]. Detecting these mutations accurately is essential for tailoring treatment strategies and monitoring patient response. However, existing methods, such as next-generation sequencing (NGS), despite their precision, are often time-consuming and expensive, making them less practical for widespread clinical use.

AmoyDx® Super-ARMS ESR1 PCR Kit

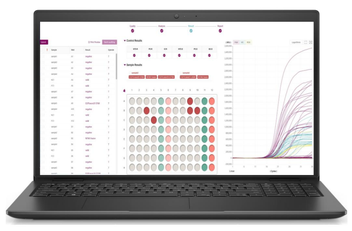
The AmoyDx® Super-ARMS ESR1 PCR Kit is a qualitative real-time PCR assay developed for the detection of 29 somatic mutations (see Table 1) in the ESR1 gene across exons 4, 5, 6, 7, and 8, using circulating free DNA (cfDNA) extracted from plasma samples. cfDNA. This assay is designed with 12-tube strips, each strip capable of testing 2 samples and leverages Super-ARMS technology, an enhanced version of traditional ARMS-PCR. This technology improves sensitivity through optimized primer and probe design, enabling efficient amplification of mutant sequences by ensuring the 3’ base of the primer matches the mutation. This facilitates the detection of low-abundance variants, which is critical for identifying clinically relevant mutations present in small quantities.

Table 1: Product Design of AmoyDx® Super-ARMS ESR1 PCR Kit

	Tube1	Tube2	Tube3	Tube4	Tube5	Tube6
FAM	External Control	p.D538G p.Y537S p.Y537N p.L536P	p.Y537C p.L536R	p.L536H p.H524L p.L549P p.Y537H	p.L536Q p.V534E p.Y537D p.D538Y p.L536K	p.D538N p.L536G
VIC	/	Internal Control	Internal Control	Internal Control	Internal Control	Internal Control
ROX	External Control	p.E380Q	p.S463P	p.G442R	p.L469V	p.V418E p.L379I
CY5	External Control	/	/	p.L370F p.E380K	p.V422del	p.G344D p.H356D p.H356Y
Limit of Detection	/	0.2%	0.5%	0.4%-0.6%	0.2%-1%	0.4%-2%



Streamlined Workflow

DNA Extraction	PCR Setup	Real-Time PCR RUN	Data Analysis
			
> 2 mL of plasma sample	Ready-to-use reagent	Open PCR devices	ARAS Automated
3 hours	10 min	2 hours	1-2 min

Evaluation Sample (Seraseq® ctDNA ESR1 Reference)

The Seraseq® ctDNA ESR1 Mix is intended for use with molecular testing assays that identify variants present in circulating tumor DNA (ctDNA) present in the blood. Seraseq ctDNA ESR1 Mutation Mix AF1% contains 22 that are associated with ctDNA monitoring and are predominantly druggable mutations (see Table 2). The product is formulated to simulate ctDNA fragment sizes with a peak between 150-220 bp. Variant allele frequency (VAF) and copy gain, is confirmed by digital PCR. VAF is also measured by NGS as reported in the batch-specific TPR.

Table 2-1: Variants covered by Super-ARMS ESR1

#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type
1	ESR1	c.1613A>G	D538G	SNV
2		c.1138G>C	E380Q	SNV
3		c.1607T>C	L536P	SNV
4		c.1609T>A	Y537N	SNV
5		c.1610A>C	Y537S	SNV
6		c.1607T>G	L536R	SNV
7		c.1387T>C	S463P	SNV
8		c.1610A>G	Y537C	SNV
9		c.1607T>A	L536H	SNV
10		c.1607_1608delinsAG	L536Q	INDEL
11		c.1609T>G	Y537D	SNV

Table 2-2: Variants **NOT** covered by Super-ARMS ESR1

#	Gene	Nucleic Acid Change	Amino Acid Change	Variant Type
12	ESR1	c.1610_1615dupATGACC	D538_L539insHD	INDEL
13		c.1625A>G	E542G	SNV
14		c.1607_1608delinsAT	L536H	INDEL
15		c.1603C>A	P535T	SNV
16		c.1608_1609delinsTA*	Y537N	INDEL
17		c.1610_1611delinsCA	Y537S	INDEL
18		c.1609_1610delinsAG	Y537S	INDEL
19	PIK3CA	c.1624G>A	E542K	SNV
20		c.1633G>A	E545K	SNV
21		c.3140A>G	H1047R	SNV
22		c.3203dupA	p.N1068Kfs*5	INDEL

** Possible to be detected by the kit.*

AmoyDx® Super-ARMS ESR1 Performance Evaluation

Method

The sensitivity of the kit was evaluated using commercially available Seraseq ctDNA ESR1 reference materials with variant allele frequencies (VAFs) of 0.5%, 0.2%, 0.1%, 0.05% and 0%. Testing was performed at total DNA inputs of 60 ng, 30 ng and 10 ng, corresponding to 10 ng, 5 ng and 1.67 ng per reaction tube, respectively (6 tubes per input amount). Each VAF and input combination was analyzed in triplicate on SLAN-96S instrument (see Table 3).

Table 3: Experiment Design and Evaluation Parameters

VAF (%)	Input 1 (ng)	Input 2 (ng)	Input 3 (ng)	Instrument	Replicate
0.5	10	30	60	SLAN-96S	3
0.2	10	30	60		3
0.1	10	30	60		3
0.05	10	30	60		3
0	10	30	60		1

Result

Analytical Sensitivity Verification:

- At an input of **10 ng** DNA, the Limit of Detection (LoD) was verified to be between **0.2%-0.5%** VAF.
- At an input of **30 ng** DNA, the Limit of Detection (LoD) was verified to be between **0.05%-0.2%** VAF.
- At an input of **60 ng** DNA, the Limit of Detection (LoD) was verified to be between **0.05%-0.1%** VAF.

Table 4: Analytical Sensitivity Verification of Super-ARMS ESR1 at **10 ng, 30 ng** and **60 ng** input

Tube	Signal	Amino Acid Change	Nucleic Acid Change	Verified LoD	10 ng input	30 ng input	60 ng input
2	FAM	p.D538G p.Y537S p.Y537N p.L536P	c.1613A>G c.1610A>C c.1609T>A c.1607T>C	0.5%	3/3	3/3	3/3
				0.2%	3/3	3/3	3/3
				0.1%	2/3	3/3	3/3
				0.05%	1/3	3/3	3/3
	ROX	p.E380Q	c.1138G>C	0.5%	3/3	3/3	3/3
				0.2%	1/3	3/3	3/3
				0.1%	1/3	1/3	3/3
				0.05%	0/3	1/3	1/3
3	FAM	p.Y537C p.L536R	c.1610A>G c.1607T>G	0.5%	3/3	3/3	3/3
				0.2%	3/3	3/3	3/3
				0.1%	2/3	3/3	3/3
				0.05%	0/3	2/3	2/3
	ROX	p.S463P	c.1387T>C	0.5%	3/3	3/3	3/3
				0.2%	2/3	3/3	3/3
				0.1%	1/3	2/3	3/3
				0.05%	2/3	1/3	2/3
4	FAM	p.L536H	c.1607T>A	0.5%	3/3	3/3	3/3
				0.2%	2/3	3/3	3/3
				0.1%	2/3	3/3	3/3
				0.05%	1/3	2/3	3/3
5	FAM	p.L536Q p.Y537D	c.1607_1608delinsAG c.1609T>G	0.5%	3/3	3/3	3/3
				0.2%	2/3	3/3	3/3
				0.1%	1/3	3/3	3/3
				0.05%	1/3	1/3	2/3

Note: The claimed LoD was established based on an input amount of 30 ng of cfDNA.

Analytical Specificity Verification:

Analytical specificity was assessed using Seraseq ctDNA ESR1 Mix WT. 100% specificity was demonstrated for all multiplex assays across 60 ng, 30 ng and 10 ng total DNA inputs (see Table 5).

Table 5: Analytical Specificity Verification of Super-ARMS ESR1

Gene	Tube	Input (ng)	Result
ESR1	2-6	10	0/1
ESR1	2-6	30	0/1
ESR1	2-6	60	0/1

Conclusion

The AmoyDx® Super-ARMS ESR1 PCR Kit exhibits high sensitivity and specificity in detecting 11 clinically prevalent ESR1 mutations within the Seraseq ESR1 reference panel. Utilizing ultrasensitive Super-ARMS technology, this assay achieves a limit of detection (LOD) of 0.2% with a 30ng DNA input, offering superior detection sensitivity compared to conventional qPCR methods. It provides laboratories with a user-friendly solution compatible with standard molecular equipment. The platform surpasses next-generation sequencing (NGS) in operational simplicity, cost-effectiveness, and rapid turnaround time (TAT), making it an efficient and reliable choice for ESR1 mutation detection.

[1] Nagaraj G, et al. Adv Ther 2021;38:109–36, [2] Hermida-Prado F, et al. Cancer Res 2021;81:537–8, [3] Toy W, et al. Cancer Discov 2017;7:277–87, [4] Jeselsohn R, et al. Curr Oncol Rep 2017;19:35, [5] Jeselsohn R, et al. Nat Rev Clin Oncol 2015;12:573–83, [6] Najim O, et al. Biochim Biophys Acta Rev Cancer 2019;1872:188315, [7] De Santo I, et al. Cancers 2019;11:1894