



Assay Technologies Ltd.



APIS ESR1 Mutations Kit

The APIS ESR1 Mutations Kit is an advanced qPCR assay for the sensitive and precise detection of mutations within the oestrogen receptor gene. The APIS ESR1 Mutations Kit is a qualitative test, detecting eleven *ESR1* mutations across three exons: exon 5 (E380Q), exon 7 (S463P) and exon 8 (P535H, L536R, L536Q, L536H, L536P, Y537C, Y537S, Y537N and D538G).

The APIS ESR1 Mutations Kit includes mutation-specific probes to enable highly sensitive detection of the target mutations. Additionally, it uses PCR clamp and blocker technology, which ensures specific amplification of the mutant sequence, even in the presence of a high wild-type (WT) background.



Benefits of Using APIS ESR1 Mutations Kit

- 1 **Wide target coverage** – the kit has been designed to cover 11 *ESR1* mutations
- 2 **High specificity** – includes clamp and blocking technology to ensure no wild-type detection
- 3 **High sensitivity** – mutations are detected at $\leq 1\%$ MAF and $\leq 0.5\%$ MAF in high WT background
- 4 **Easy to use** – our assays are designed to be user-friendly, with a simple protocol. The reagents have been optimised for precise and sensitive detection in human DNA.

Analytical Sensitivity in Contrived Samples

The APIS ESR1 Mutations Kit detects 11 *ESR1* mutations across three exons.

Contrived samples of 0.008–1.6% mutant allele frequency (MAF) were prepared with a total copy of 5000 per reaction. A total of 24 replicates were generated per target, across two instruments and two kit lots. The Limit of Detection (LoD) is based on the highest MAF with 95% correct calls observed for each target in the 24 replicates. The claimed LoD for the targets in % MAF is $\leq 1\%$ for all targets (Table 1).

Table 1. 11 *ESR1* mutations detected in the APIS *ESR1* Mutations Kit. LoD in % MAF and copies (cp). The mutant copies at LoD and WT copies at LoD describe the copies of each DNA fragment in the contrived LoD samples.

Exon	Mutations to be Detected	Nucleic Acid Change	COSMIC ID	LoD (%MAF)	Mutant cp at LoD	WT cp at LoD	
5	E380Q	c.1138G>C	COSM3829320	1.00%	50	4950	
7	S463P	c.1387T>C	COSM4771561	0.08%	4	4996	
8	P535H	c.1604C>A	COSM4944018	0.40%	20	4980	
	L536R	c.1607T>G	COSM4774826	0.70%	35	4965	
	L536Q	c.1607_1608delinsAG (TC>AG)	COSM4766050	0.80%	40	4960	
	L536H		c.1607T>A	COSM6843697	0.80%	40	4960
	L536P		c.1607T>C	COSM6906109	0.90%	45	4955
	Y537S		c.1610A>C	COSM1074639	0.10%	5	4995
	Y537N	c.1609T>A	COSM1074635	0.20%	10	4990	
	Y537C	c.1610A>G	COSM1074637	0.40%	20	4980	
D538G	c.1613A>G	COSM94250	0.40%	20	4980		

Sensitivity in High WT background

To assess kit performance in high WT background, samples were contrived with WT and mutant DNA at 0.5% MAF, with a total DNA copy number of 10,000 or 50,000 per reaction. Table 2 shows the mutations for which the APIS *ESR1* Mutations Kit achieves sensitivity of $\leq 0.5\%$ MAF in a high WT background.

Table 2. *ESR1* Mutations detected at $\leq 0.5\%$ MAF in high WT background. The Mutant DNA and WT copies in the contrived 0.5% MAF samples are shown.

Target	LoD (%MAF)	Mutant DNA copies at 0.5% MAF	WT DNA copies in 0.5% MAF sample
D538G*	$\leq 0.5\%$	50	9,950
Y537S*	$\leq 0.5\%$	50	9,950
Y537C*	$\leq 0.5\%$	50	9,950
Y537N*	$\leq 0.5\%$	50	9,950
L536X.1 (H)	$\leq 0.5\%$	50	9,950
L536X.1 (Q)	$\leq 0.5\%$	50	9,950
E380Q	$\leq 0.5\%$	250	49,750
P535H*	$\leq 0.5\%$	50	9,950

* Targets detected with 0.5% MAF sensitivity in samples with both 10,000 and 50,000 total copies.

Supporting Data for the APIS ESR1 Mutations Kit Linearity

An 8-level dilution series of DNA fragments specific to each mutation were used to evaluate PCR efficiency and R² values (Table 3 and Figure 1). Three replicates were assessed at each level to determine linearity across the target range from 5 to 10,000 DNA copies. Linear regression was plotted for each mutation, representative D538G mutation linear regression plot shown.

Table 3. Linearity analysis of each target in the APIS ESR1 Mutations Kit.

Target	Slope	Efficiency (%)	R ²	Dynamic Range (DNA copies)
D538G	-3.43	95.6	1.00	5–10,000
S436P	-3.26	102.5	0.99	5–10,000
Y537S	-3.64	88.2	0.99	5–10,000
Y537C	-3.33	99.7	1.00	5–10,000
Y537N	-3.34	99.1	0.99	5–10,000
L536X.1 (H)	-3.41	96.5	0.99	5–10,000
L536X.1 (Q)	-3.41	96.6	1.00	5–10,000
E380Q	-3.32	99.9	0.99	5–10,000
P535H	-3.35	98.7	1.00	5–10,000
L536X.2 (P)	-3.39	97.4	0.99	5–10,000
L536X.2 (R)	-3.35	98.9	1.00	5–10,000
Reference	-3.33	99.6	0.98	5–10,000

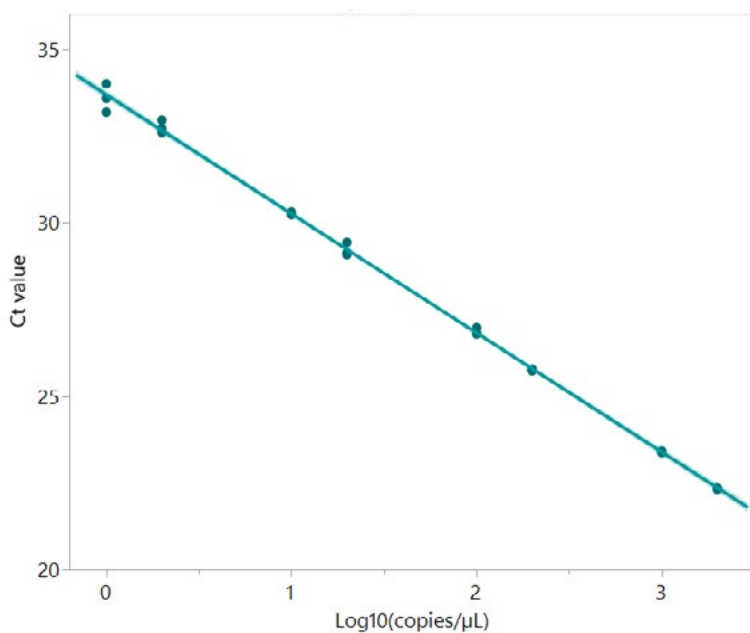


Figure 1. Linear regression of the D538G mutation across the 8-level dilution series.

APIS ESR1 Mutations Kit Specifications

The kit has undergone testing as part of APIS' unique assay development pipeline, which includes *in silico* and wet laboratory testing. As a result, researchers can have confidence in the accuracy, sensitivity, specificity, and reliability of the Kit. The qPCR assay's efficiency and linearity were assessed using contrived synthetic DNA samples.

Format	10 Tubes: 6 PP mixes (Primer-Probes), 2 Enzyme mixes, 1 Positive Control, 1 Negative Control
PCR Method	qPCR
Instrument	Any qPCR instrument with compatible cycling and emission detection capabilities
Probe Modifications	5' FAM™/3' BHQ®1 5' HEX™/3' BHQ®1
No. of Samples	Standard kit is sufficient for 24 samples (20 µL reaction), including pipetting overage
Concentration	4X PP mix 2X Enzyme mix
Sample Type	DNA (e.g. cell-free DNA or DNA extracted from FFPE tissue)

APIS ESR1 Mutations Kit Ordering Information

Assay Name	Test Type	Kit Size	Catalogue Number	Price
APIS ESR1 Mutations Kit	RUO	24 samples plus controls	02201 (distributed by APIS)	Available upon request
APIS ESR1 Mutations Kit	RUO	24 samples plus controls	02202 (distributed by Biocartis)	Available upon request

Please visit the product web page, for the list of countries that Biocartis distributes the APIS ESR1 Mutations Kit. APIS is the distributor for all other countries. Data and conclusions provided in this leaflet have not been validated by Biocartis NV.

International distributor:



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All scientific and analytical information in this brochure comes from the APIS ESR1 Mutations Kit Handbook ART0067.

The APIS ESR1 Mutations Kit is intended for Research Use Only (RUO). Not for use in medical or clinical diagnostic procedures.