



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 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** – all mutations are detected at $\leq 1\%$ MAF (Mutant Allele Frequency)
- 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.

Assay Mutations and 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 mutant allele frequency 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).

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	

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.

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 R^2 values (Table 2 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.

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

Table 2. Linearity analysis of each target in the APIS ESR1 Mutations Kit.

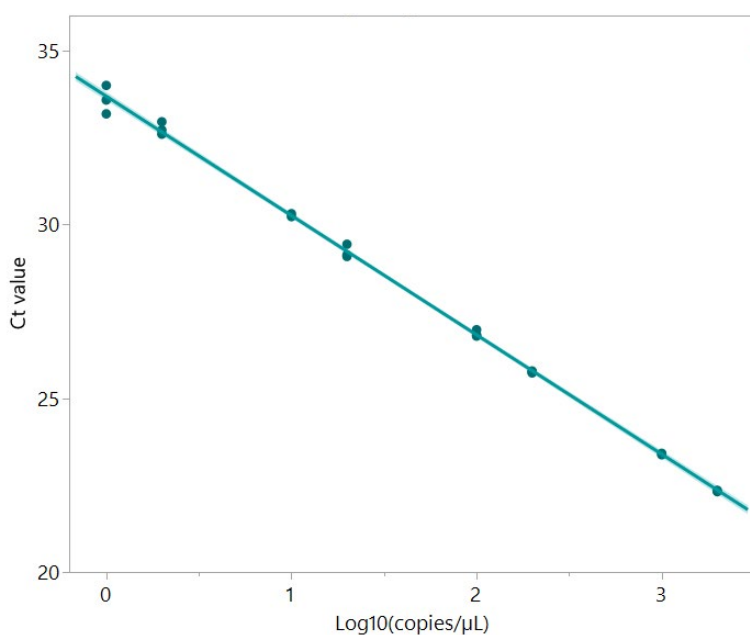


Figure 1. Linear regression of the D538G mutation across the 8-level dilution series.

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 Buffer 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' BHQ1 5' FAM/3' BHQ1
No. of Samples	Standard kit is sufficient for 24 samples (20 µL reaction), including pipetting overage
Concentration	4X PP mix 2X Buffer mix
Sample Type	DNA extracted from plasma (cfDNA)

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.

International distributor:



Biocartis

Phone: +32 (0) 15 632 600

Address: Generaal De Wittelaan 11B, 2800 Mechelen, Belgium

Email: info@biocartis.com

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.