



Assay Technologies Ltd.

APIS ESR1 Mutations Kit - Technical Sheet

Kit Overview

The APIS ESR1 Mutations Kit is an advanced qPCR assay for the sensitive and precise detection of mutations within the oestrogen receptor gene. The 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 kit includes mutation-specific probes to enable highly sensitive detection of the target mutations. Additionally, it utilises PCR clamp and blocker technology, which ensures specific amplification of the mutant sequence, even in the presence of a high wild-type background.

Intended Use

The APIS ESR1 Mutations Kit is a Research Use Only (RUO) real time polymerase chain reaction (qPCR) assay for the detection of ESR1 mutations in deoxyribonucleic acid (DNA).

Features of the Assay

- Wide target coverage – the kit has been designed to cover 11 different ESR1 mutations
- High specificity - includes clamp and blocking technology to ensure no wild-type detection
- High sensitivity – all mutations are detected at $\leq 1\%$ MAF (Mutant Allele Frequency)
- 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.

Minimum Sample Requirements

The assay is designed for use with DNA samples, for example, extracted cell-free DNA (cfDNA). It is recommended to extract samples using a cell-free-specific kit and to use the sample without dilution. 30 μ L of sample is required to assess all mutations detected by the kit.

Turnaround Time

8 samples in <2 hours from PCR set up to results generation.

Result Reporting

Ct values should be used to guide the mutation status (positive or negative). Samples in which a Ct value is reported, are positive for that target's mutation. If no Ct is reported, the mutation status is negative.

If a positive Ct value is reported for L536X.1 or L536X.2, the ESR1 mutation present in the sample is either L536R, L536P, L536H or L536Q.

Performance data

Assay Mutations and Analytical Sensitivity in Contrived Samples

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

Contrived samples of a varying 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. 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	0.40%	20	4980
	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

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 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.

Table 2. 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

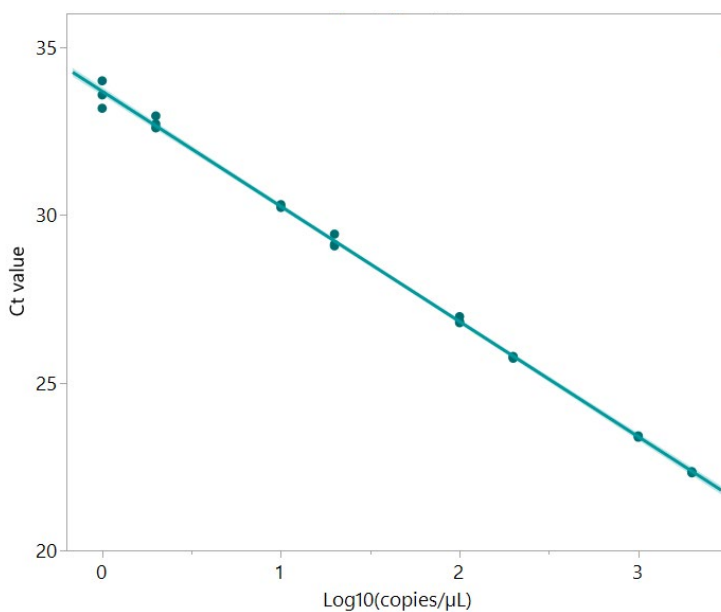


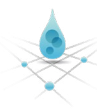
Figure 1. Linear regression of the D538G mutation across the 8-level dilution series.

APIS

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To order the APIS ESR1 Mutations Kit or to learn more about how our assay can elevate your breast cancer research capabilities, please contact APIS or Biocartis using the details below.

International distributor:



Distributed by
BIOCARTIS

Biocartis

Phone: +32 (0) 15 632 600

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

Email: info@biocartis.com



Ordering Information

Product 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.

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