



APIS MET Alterations Kit

The APIS MET Alterations Kit is a unique real-time PCR (RT-qPCR) test simultaneously detecting both *MET* exon 14 skipping mutation (*MET* Δ ex14) and *MET* gene overexpression, using RNA extracted from FFPE non-small cell lung cancer (NSCLC) tissue or liquid biopsy (cfRNA).

Advantages of the APIS MET Alterations Kit



Simultaneous detection of *MET* overexpression and exon 14 deletion



Simple protocol compatible with most qPCR instruments

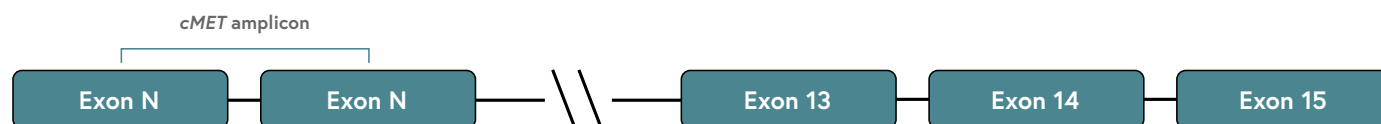


Designed for tumour tissue (FFPE) or liquid biopsy

Alterations Detected by the APIS MET Alterations Kit

The assay is designed to detect the relative expression of the wild type *cMET* transcript, and the RNA product of mutations in *MET* that lead to the exon-skipping (deletion) of exon 14 (*MET* Δ ex14 amplicon).

A - *MET* Overexpression



B - *MET* Exon 14 Deletion

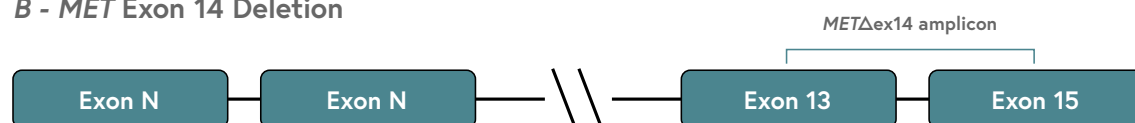


Figure 1. Alterations detected by the APIS MET Alterations Kit (A) *MET* overexpression (*cMET*) (B) *MET* exon 14 skipping mutation (*MET* Δ ex14)

APIS *MET*Δex14 Assay Sensitivity (Limit of Detection)

The Limit of Detection (LoD) of *MET*Δex14 assay was estimated by testing synthetic RNA fragments encoding *MET*Δex14 at varying concentrations, in a background of 10ng healthy lung total RNA (containing approx. 2100 copies of the wild type transcript).

The LoD was defined as the lowest number of *MET*Δex14 copies with a 100% detection rate (8/8 replicates).

The LoD for *MET*Δex14 was established at 25 copies (1.17% MAF).

Copies of <i>MET</i> Δex14	%MAF	Wildtype DNA copies at LoD	Mean Ct	Hit rate
80	3.75	2134	33.97	8/8
69	3.23	2134	34.90	8/8
58	2.72	2134	35.07	8/8
47	2.20	2134	35.51	8/8
36	1.69	2134	35.64	8/8
25	1.17	2134	37.15	8/8
14	0.66	2134	38.26	3/8
3	0.14	2134	40.00	0/8

Table 1. LoD estimation for the *MET*Δex14. The estimated mutant allele frequency (%MAF) for the deletion for each copy number is indicated. LoD of 25 copies shown in bold.

Limit of Blank (LoB): Determined by testing negative RNA samples derived from cell-line, and total human lung RNA. For each target, the overall rate of correct sample interpretation was ≥98%.

Detection of Relative *cMET* Expression

The APIS MET Alterations Kit allows for the detection of *cMET* relative gene expression. The expression of *cMET* was measured in three cell lines with pre-characterised transcript numbers (Figure 2).

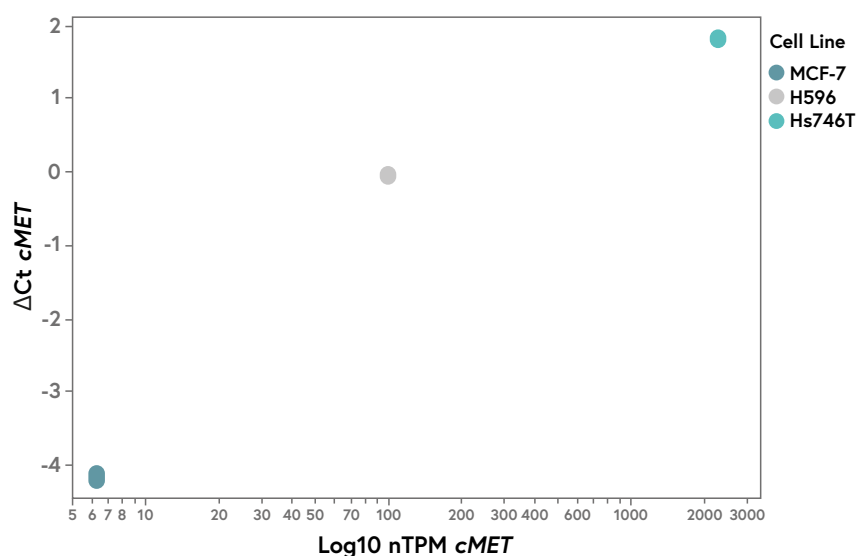


Figure 2. *cMET* ΔC_t values from three *cMET* characterised cell lines, MCF-7, H596 and Hs746T plotted against Log10 nTPM (normalised transcripts per million). Hs746T harbours both amplified *cMET* and the *MET* Δ ex14 mutation, while H596 and MCF-7 are negative for *MET* overexpression. Results generated using QS5™ Dx instrument.

APIS MET Alterations Kit Dynamic Range

The dynamic range of *cMET* and the two reference genes (*IPO8* and *UBXN4*) was determined using a dilution series of healthy lung RNA. Synthetic RNA in a wild type background of ~2200 copies was used to determine the dynamic range of *MET* Δ ex14.

Table 2. Linearity analysis of each target in the APIS MET Alterations Kit

Target	Slope	Efficiency (%)	R ²	Dynamic Range (RNA or copies)
<i>IPO8</i>	-3.33	99.66	0.998	38-0.3ng
<i>UBXN4</i>	-3.26	102.65	0.992	38-0.15ng
<i>cMET</i>	-3.43	95.67	0.991	38-0.15ng
<i>MET</i> Δ ex14	-3.69	87.92	0.990	2750-13 copies

APIS MET Alterations Kit Specifications

The APIS MET Alterations Kit contains two reference genes which allow for normalisation of assay input and monitor for assay set up. Positive and Negative Controls (PC and NTC) which monitor for both assay set up and reagent performance are also supplied with the kit.

Format	7 Tubes: 2 PP Mixes (Primer-Probes), 2x Enzyme Mix, 1 Buffer Mix, 1 Positive Control, 1 Negative Control
PCR Method	RT-qPCR
Instrument	Any qPCR instrument with compatible cycling and emission detection capabilities
Probe Modifications	5' FAM 5' HEX
No. of Samples	Standard kit is sufficient for 24 samples (20µL reaction) in duplicate, including pipetting overage
Concentration	1X Enzyme Mix 1X Buffer Mix Variable PP Mix
Sample Type	RNA extracted from FFPE NSCLC tissue or cell-free RNA (cfRNA)

APIS MET Alterations Kit Ordering Information

Assay Name	Test Type	Kit Size	Catalogue Number	Price
APIS MET Alterations Kit	RUO	24 samples in duplicate plus controls	03001	Available upon request

**The APIS MET Alterations Kit is a Research Use Only (RUO) product.
Not for use in diagnostic procedures.**