



## Positive Control for ICEme Kit

The positive control supplied with this kit consists of a mixture of digitally verified cell lines from Horizon Discovery.

The positive control is included in the kit to provide quality control checks at specific steps in the MX PCR procedure and MX-ICP procedure. For the MX PCR Pre-Amplification Protocol, this control provides a means to ensure that the MX PCR Master Mix is correctly prepared and amplification is functioning properly. For the MX-ICP Enrichment Protocol(s), this control provides a means to ensure that the MX-ICP Master Mix is correctly prepared and the enrichment of specific mutations in the amplicon of interest is functioning properly. No Template Controls (where water is added in place of template DNA) are also required to check for possible contamination of the MX PCR and the MX-ICP Master Mix(es).

### Positive Control

- Mutations in Positive Control Mixture are listed in Table 1
- Values are the digitally verified ratios of each mutation in any given aliquot
- High ratio mutations, asterisked in Table 1, can be detected after MX PCR
- Other low ratio mutations in Table 1 can only be detected after MX-ICP enrichment.

### Use of Positive Control

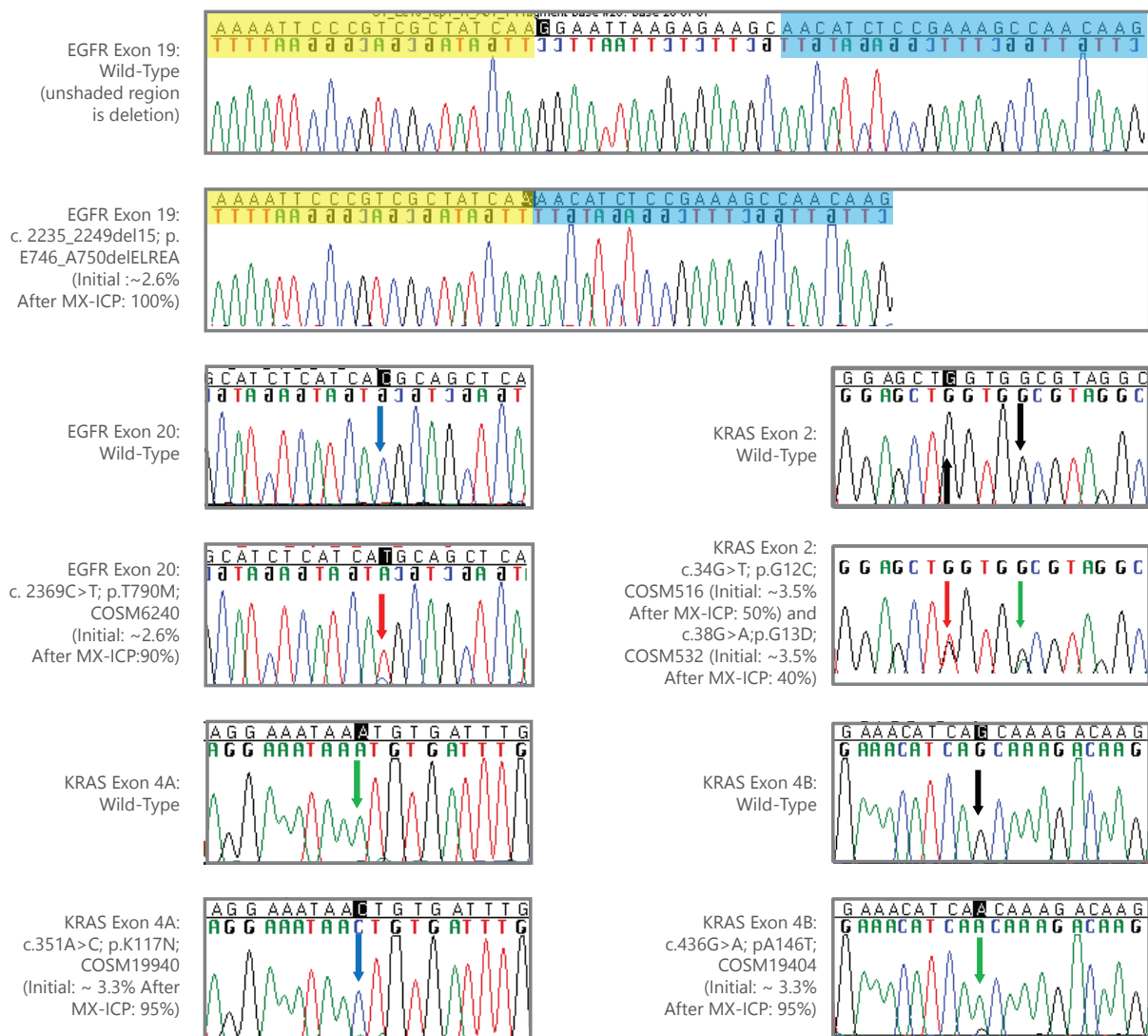
Effective use of the ICEme Kit depends upon successful completion of a number of steps:

- MX PCR amplification that must result in the production of specific, uniform-sized DNAs
- Quality of the initial sample is critical
- Assays where DNA does not meet the quality and quantity criteria is not recommended
- Detection of the high ratio mutations asterisked in Table 1 can be used as a QC step for the MX PCR reaction.
- Examples shown in Figure 1 are Sanger sequencing electropherogram examples of positive controls both before and after MX-ICP.

**Table 1. Postive Control Mixture**

Gene	Variant	Expected Ratio (ddPCR)
BRAF Ex 15*	V600E	19.2
EGFR Ex 18*	G719S	24.9
EGFR Ex 12	S492R	2.7
EGFR Ex 19	ΔE746 - A750	2.6
EGFR Ex 21	L858R	2.5
EGFR Ex 21	L861Q	2.3
EGFR Ex 20	T790M	2.6
KRAS Ex 2	G13D	3.5
KRAS Ex 2	G12C	3.5
KRAS Ex 3	Q61H	3.5
KRAS Ex 4A	K117N	3.3
KRAS Ex 4B	A146T	3.3
NRAS Ex 2	G12D	3.4
NRAS Ex 3	Q61K	3.3
NRAS Ex 4A	K117N	3.7
NRAS Ex 4B	A146T	3.4
PIK3CA Ex 9	E545K	3.3
PIK3CA Ex 20*	H1047R	16.6

Figure1. Sanger sequencing electropherogram



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