

## **MX-ICP KRAS Exon 3 Kit Components**

The KRAS Exon 3 Kit contains the components to perform ICE COLD-PCR amplification for mutation enrichment as well as single direction Sanger sequencing for 29 total samples with controls. Recommended storage conditions are listed in Table 1.

Table 1: Kit components with recommended storage conditions.

Reagent, ICE COLD-PCR	Lid Color	Color of Label	Volume Total (μL)	Storage (°C)
Primers/RS-Oligo Mix	Clear	Blue	180	-20
2X Polymerase Master Mix	Clear	Green	900	-20
Wild Type Control	Clear	White	18	-20
1% Q61H (A>C) Control	Clear	Red	18	-20
Reagent	Lid Color	Color of Label	Volume Total (μL)	Storage (°C)
Sanger Sequencing Primer, 10 μM	Clear	Orange	40	-20

The kit contains enough reagents to perform 32 total analyses. For optimal usage of the kit, it is suggested that 29 samples are run per batch along with one set of controls.

This kit was designed specifically to detect the following mutations:

- 1. c.175G>A; p.A59T
- 2. c.183A>C; p.Q61H
- 3. c.183A>T; p.Q61H
- 4. c.181C>A; p.Q61K
- 5. c.182A>T; p.Q61L
- 6. c.182A>G; p.Q61R

## **Reagents Required but not Supplied**

1. Molecular Biology Grade Water: Thermo Fisher Catalog # AM9937

## **Primary Sample Collection, Handling and Storage**

This Kit can be used with the following:

- DNA extracted from formalin-fixed paraffin-embedded tumor samples (FFPE slides & blocks) or fine needle aspirations (FNAs)
- Circulating free DNA (cfDNA) from plasma or serum
- DNA isolated from other body fluids

For optimal DNA extraction from FFPE, the tissue should be fixed in neutral buffered formalin for 14–24 hours, placed in ethanol and then embedded in paraffin following standard histological practices. Tumor biopsies are a heterogeneous mixture of tumor cells and non-tumor cells. In addition the tumor itself is a heterogeneous mixture of tumor cells with mutations and tumor cells without mutations. Because these somatic mutations may not be evenly distributed throughout the tumor, the resultant mutational analysis of different sections from the same tumor may be different. To increase the probability of detecting a mutation, DNA from the tumor region of the tissue should be isolated by scraping only the tumor area from the glass slide using a fresh, sterile scalpel for each new slide. It is recommended that at least two independent analyses are performed for each sample.