CERTIFIED SOCIETY
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CE

Identification of mutation of codon 12 and codon 13 of K-Ras protein

AMPLI set K-Ras Real Time Cat.n.1.428RT

KRAS mutations has been detection in almost 30% of solid tumours, and it has been shown to predict response to target therapies. The most frequent alterations of KRAS are somatic missense mutations in the gene that lead to single amino acid substitutions. The mutations detected most frequently are in codons 12 (about 80% of all reported KRAS mutations: GGT-AGT; GGT-TGT; GGT-CGT; GGT-GAT; GGT-GTT; GGT-GCT) and 13 (about 17%: GGC-GAC). Mutations in codon 61 has also been reported but this alteration account for a minor proportion (1-4%) of KRAS mutations. These mutations result in proteins that are permanently in the active GTP-bound form due to defective intrinsic GTPase activity and resistance to GTPase-activating proteins (GAPs). Unlike wild-type proteins which are inactivated after a short time, the aberrant KRAS are able to continuously activate signaling pathway in the absence of any upstream stimulation of receptors.
The most common strategy for the detection of KRAS mutations is the direct sequencing. This method has a sensitivity of 10-30%, depending on which technique is used, pyrosequencing or Sanger method.
The kit allows the identification of mutations in codon 12 and codon 13 by allele-specific PCR and detection in REAL-TIME PCR, thanks to the presence of a probe labeled with the fluorophore FAM.
This kit uses allele-specific PCR (ARMS-PCR Amplification Refractory Mutation System - Polymerase Chain Reaction), which allows sensitive detection allele wild-type (normal) and mutant detection in Real TimePCR. The allele-specific PCR, compared to other methods (sequencing, RFLP etc), allows to identify the mutation even when present in a small percentage of cells (1-2% of mutated cells sensitivity, specificity 99%) and identify specifically the mutations in codon 12 (G12X) and codon 13 (G13D), respectively.
The assay also uses an internal amplification control to verify the quality and quantity of genomic DNA of each sample.

Principle of the method: a) extraction of genomic DNA from tissue or from paraffin embedded tissue; b) Real Time PCR
Applicability of genomic DNA extracted and purified from biological samples, fresh tissue and paraffin.
Number of Tests: 96.

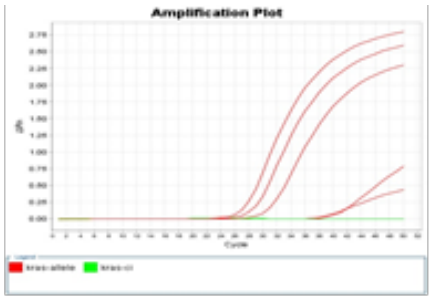
KIT CONTAINS AND STORAGE

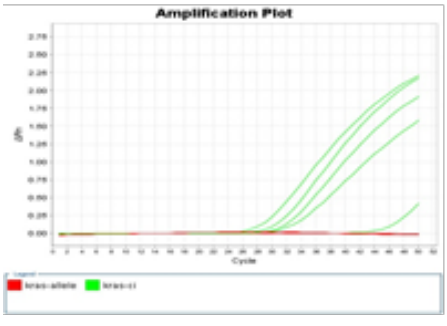
AMPLIFICATION	
Master Mix 2X	-20°C
MIX primer probe 20X PCR K-ras **G12S**	-20°C
MIX primer probe 20X PCR K-ras **G12R**	-20°C
MIX primer probe 20X PCR K-ras **G12C**	-20°C
MIX primer probe 20X PCR K-ras **G12D**	-20°C
MIX primer probe 20X PCR K-ras **G12A**	-20°C
MIX primer probe 20X PCR K-ras **G12V**	-20°C
MIX primer probe 20X PCR K-ras **G13D**	-20°C
MIX primer probe 20X **Internal Control**	-20°C
Controllo WT	+4°C
Controllo Eterozigote (G12C)	+4°C
H_2O sterile RNase/DNase FREE	-20°C

Stability: more than 18 months if properly stored.

INTERPRETATION OF RESULTS

The analysis of the results must be made through the analysis of the amplification curves (AMPLIFICATION PLOT).
The probe present in the PCR mix for each specific mutation is labeled with the fluorophore FAM. Ct values comprised of 24 to 30 indicate the presence of mutation (curves 1, 2, 3).
The probe that allows the revelation of internal control is labeled with the fluorophore Joe (green curves in (kras-us). The amplified must be present in all samples, regardless of the presence or absence of the mutation in the Kras gene.





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