CERTIFIED COMPANY
UNI EN ISO 9001, UNI EN ISO 13485

DETECTION of MUTATIONS of N-RAS PROTEIN'S CODON 12, 13 e 61

AMPLI-set-N-Ras Real Time Cat. n. 1.433RT

N-RAS mutations have been observed in about 25 % of cancers and they can predict the outcome of focused therapies. The most frequent N-RAS mutations, involved in a malignant transformation, are the point ones localized in codon 12 (GGT-AGT; GGT-TGT; GGT-CGT; GGT-GAT; GGT-GTT; GGT-GCT) and, less frequently, in codon 13 (GGT-AGT) and 61 (CAA-AAA; CAA-CGA). These amino acids' residues play a key role in bonding to GTP. These point mutations engender the oncogenetic protein p21 Ras, able to resist to GTP hydrolysis and constitutive activated .
The most common strategy used for the N-RAS mutations' identification is the conventional PCR sequencing. This approach has a sensitivity of 10-30% depending whether pyro sequencing or Sanger method has been used.
This kit allows the mutations' detection of codon 12, 13 and 61 through allele-specific PCR and Real-Time PCR, thanks to a probe fluorofore FAM labelled.
The assay is based on allele-specific PCR (ARMS-PCR, Amplification Refractory Mutation System-Polymerase Chain Reaction). This technique allows a sensitive detection of wild-type allele (healthy) and mutated one with Real Time PCR detection. The allele-specific PCR, compared to other methods (sequencing, RFLP), enables to detect the mutation, even when it is present only in a small percentage of cells (sensitivity 1-2% of mutated cells; specificity 99%), and to specifically identify the codons' mutations.
The kit is provided with an amplification internal control for verifying, either, the quality and the quantity of genomic DNA of every sample.

Principle assay: A) extraction of genomic DNA from tissue o paraffinated tissue B) amplification and detection by Real Time PCR.
Applicability: tissue genomic DNA, paraffinated tissue genomic DNA.
Number of tests: 16x8

REAGENTS AND STORAGE

AMPLIFICATION	
Master Mix 2X	-20°C
MIX primer probe 20X PCR N-ras **G12S**	-20°C
MIX primer probe 20X PCR N-ras **G12R**	-20°C
MIX primer probe 20X PCR N-ras **G12C**	-20°C
MIX primer probe 20X PCR N-ras **G12D**	-20°C
MIX primer probe 20X PCR N-ras **G12A**	-20°C
MIX primer probe 20X PCR N-ras **G12V**	-20°C
MIX primer probe 20X PCR N-ras **G13S**	-20°C
Mix primer probe 20X PCR N-ras **Q61K**	-20°C
Mix primer probe 20X PCR N-ras **Q61R**	-20°C
MIX primer probe 20X **Internal Control**	-20°C
Control WT	+4°C
Heterozigous control	+4°C
H_2O sterile RNase/DNase FREE	-20°C

Stability over 18 months is correctly stored.

References:
Thyroid. 2014 1275-81.
Clin Cancer Res. 19 1902-12. 2013
Lancet Oncol. 11 753-62 2010
Genes Chrom Cancer. 2011 307-12.

INTERPRETATION OF RESULTS

Result interpretation has to be performed trough amplification curves analysis (AMPLIFICATION PLOT).
The probe in the mix PCR is specific for every mutations and it is labelled with florochrome FAM. Ct values in the range between 24 and 30 indicate the mutation presence (curve 1, 2, 3). The probe used for internal control is labelled with flourocrome Joe, (green curve (N-ras-ci)). The amplified has to be detected in every samples, irrespective whether the N-RAS mutation is present.