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KIT FOR THE DETECTION OF KRAS G12X and G13D MUTATIONS

AMPLI KRAS COLD NESTED PCR Cat. 1.428n

K-ras mutations have been detected in approximately 30% of all human tumors and have been shown to predict response to some targeted therapies. Most of malignant KRAS mutations are point mutations localized on codon 12 (GGT-AGT; GGT-TGT; GGT-CGT; GGT-GAT; GGT-GTT; GGT-GCT) and, with less frequency, on codon 13 (GGC-GAC) and 61 These amino acid residues play a key role in the bound with GTP and these point mutations lead to the production of a oncogenic protein p21 RAS , that is resistant to hydrolysis of GTP and it is constitutively active.
The most common KRAS detection strategy consists of conventional PCR and direct sequencing. This approach has a sensitivity of 10-30% if pyrosequecing or Sanger method is used. To improve detection sensitivity we compared our conventional method with COLD-PCR (co-amplification-at-lower denaturation-temperature PCR) which selectively amplifies minority alleles. The sensitivity of COLD-PCR was determinated by assessing serial dilutions.
COLD-PCR is a simple method and does not require additional cost for instruments. The method is specific and reproducible. COLD-NESTED PCR successfully detected mutations in all samples that were positive by conventional PCR, and enhanced the ratio mutant/ wild type of 52 fold, increasing the mutation detection sensitivity to 0,8%.
The enhancement of mutation detection by COLD-PCR inversely correlated with the tumor-cell percentage in a sample .the method consists of two different amplifications after the COLD-PCR with specific primers followed by enzymatic digestion with MvaI and HaeIII enzymes to identify mutations on codon 12 and 13 respectively.
The kit allows to detect 24 samples (COLD PCR –NESTED PCR).

Principle of method: A) isolation of cfDNA, DNA from tissue, DNA from fixed tissues B) COLD PCR and Nested PCR amplification C) enzymatic digestion D) detection on agarose gel.
Applicabilty: cfDNA, DNA from tissue , DNA from fixed tissue.
Number of test: 24 test (MIX COLD PCR K-ras G12X G13D, MIX PCR K-ras G12X, MIX-PCR K-ras G13D)

REAGENTS AND STORAGE

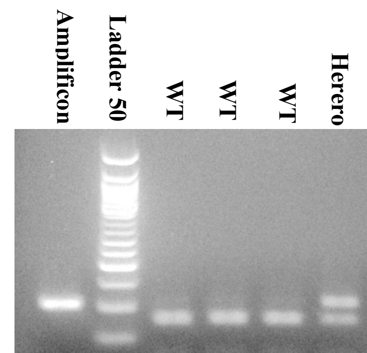
COLD PCR-NESTED PCR	
MIX COLD PCR K-ras G12X G13D	-20°C
MIX PCR K-ras G12X	-20°C
MIX PCR K-ras G13D	-20°C
H_2O sterile RNase/DNase FREE	-20°C
K-ras G12X heterozogous control	-20°C
K-ras G12X G13D wild type control	-20°C
Taq Polymerase (5U/µl)	-20°C
ENZYMATIC DIGESTION	
H_2O sterile RNase/DNase FREE	-20°C
MvaI enzyme(10 U/µl)	-20°C
Digestion BUFFER MvaI 10X	-20°C
HaeIII enzyme(10 U/µl)	-20°C
digestion BUFFER HaeIII 10X	-20°C

Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS G12X

The amplification product **K-RAS G12X** has a size of 107 bp. The following enzymatic digestion by the MvaI enzyme may lead to these results:

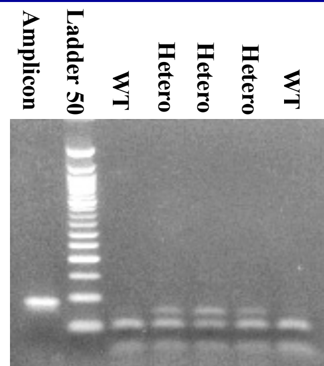
1 Wild type subject	2 heterozigous subject	3 homozigous subject
2 fragments	3 fragments	1 fragment
	107 bp	107 bp
77 bp	77 bp	
30 bp	30 bp	



ANALYSIS OF RESULTS G13D

The amplification product of **K-ras G13D** is a fragment of 89 bp. The following restiction cut by Hae III enzyme may leads to the following results:

1 Wild type subject	2 Heterozigous subject	3 Homozigous subjct
3 fragments	4 fragments	2 fragments
	69bp	69 bp
47bp	47bp	
22bp	22 bp	
20bp	20bp	20 bp



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