DETECTION of MUTATIONS of CODON 12 AND CODON 13 of K-RAS PROTEIN

AMPLI-set-K-Ras Cat. n. 1.428

The RAS-MAPK (Mitogen Activated Protein Kinase) and PI(3)K(phosphatidylinositol-3-OH kinase) signaling pathways form an intersecting biochemical network that, when mutated, leads to unrestricted cell growth.
KRAS (Kirsten Rat Sarcoma 2 viral oncogene homolog) belongs to the Ras family of oncogenes, which also includes two other genes: HRAS and NRAS. The proteins produced from these three genes are GTPases that binds to at least 3 types of effector proteins: kinases of the RAF family (including BRAF), phosphoinositide (PI) 3-kinase and members of a family of the exchange factors for the small GTPase Ral.
A diverse number of stimuli such as mitogens, hormones and neurotransmitters promote the activation of Raf kinases by first triggering increases in the levels of Ras-GTP in cells. The GTP-bound forms of Ras directly bind and thereby recruit cytosolic dimers of Raf kinases to the plasma membrane, where Raf is activated through phosphorylation by other kinases and potentially by autophosphorylation. Activated and membrane-associated Raf assembles a MAP kinase signalling complex that consists of two classes of kinases, MEK and ERK, and scaffolding proteins. The MAP kinase cascade initiates with the phosphorylation and activation of MEK by Raf and the subsequent phoshorylation and activation of ERK by MEK. Active ERK dissociates from the Raf/MEK/ERK complex and phosphorylates a number of cytoskeletal proteins, kinases and transcription factors. The functional consequences of substrate phosphorylation by ERK are dependent upon cellular context and include alterations in cellular motility and a multitude of gene expression changes that promote proliferation, differentiation, cellular survival, immortalization and angiogenesis.
Aberrant activation of this pathway, often caused by activating mutations in the composite enzymes, occurs in many tumors.
The proto-oncogene KRAS is mutated in about 15%–30% of human cancers overall (colorectal adenocarcinomas, pancreas and lung cancers, acute myelogenous leukemia and others).
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 signalling pathway in the absence of any upstream stimulation of receptors.
KRAS mutations in codons 12 and 13 appear to play a major role in the progression of colorectal cancer, while mutations in codons 12, 13 and 61 are potential biomarkers in lung cancer.
The detection of the mutations are performed carrying out two PCR with specific primers for the codon 12 and codon 13, followed by the restriction digestion by MvaI and HaeIII enzymes respectively.

Principle of assay : A) extraction of genomic DNA B) amplification C) enzymatic digestion D) detection on agarose gel
Applicability: genomic DNA extracted by fresh or paraffin-embedded tissue section.
Number of tests : 24 (48 PCR reactions)

REAGENTS AND STORAGE

AMPLIFICATION AND RESTRICTION DIGESTION	
PCR mix K-ras G12X	-20°C
PCR mix K-ras G13D	-20°C
sterile H_2O	-20°C
Taq Polymerase (5U/µl)	-20°C
MvaI enzyme (10 U/µl)	-20°C
HaeIII enzyme (10 U/µl)	-20°C
digestion BUFFER 10X for MvaI	-20°C
digesion BUFFER 10X for HaeIII	-20°C
heterozigous control	-20°C
wild type control	-20°C

Stability: over 18 months if correctly stored.

ANALYSIS OF RESULTS

G12X

100bp ampl wt wt wt et

G13D

Ampl 50bp wt et

References:
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