DETECTION OF MUTATION V600E OF B-Raf PROTEIN

AMPLI-V600E B-Raf Cat. n. 1.423

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.
BRAF is a member of the Raf family of protein kinases. 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.
Recently, activating somatic mutations in BRAF have been reported in 70% of melanomas. These mutations are also present in premalignant atypical or dysplastic nevi and may thereby implicate BRAF activation as an initiating event in tumorigenesis. BRAF mutations also occur with high frequency in papillary thyroid carcinomas, serous ovarian cancers, and colorectal serrated adenocarcinomas whereas mutations in liver, pancreas, non–small-cell lung cancer, glioma, and acute myelogenous leukemia have been detected at lower frequency.
Almost 90% of BRAF mutations are a T1799A transversion in exon 15 that results in a Val600Glu (V600E) amino acid substitution leading to constitutive kinase activation. The analysis of BRAF mutation status appears an important parameter for selecting patients for targeted therapies. The detection of the mutation is performed carrying out a PCR and a nested PCR with specific primers, followed by the restriction digestion by HpyCH4-IV

Principle of assay: A) extraction of genomic DNA
B) amplification C) direct sequencing
Applicability: genomic DNA extracted by fresh or paraffin-embedded tissue section .
Number of tests : 25

AMPLIFICATION AND RESTRICTION DIGESTION	
I PCR mix BRAF V600E	-20°C
II PCR mix BRAF V600E nested	-20°C
H2O sterile RNase/DNase FREE	-20°C
Taq Polymerase-I PCR (5U/□l)	-20°C
Taq Polymerase-II PCR (5U/□l)	-20°C
HpyCH4-IV ENZYME (10U/□l)	-20°C
digestion BUFFER 10 X	-20°C
Normal control BRAF V600E (TT)	-20°C

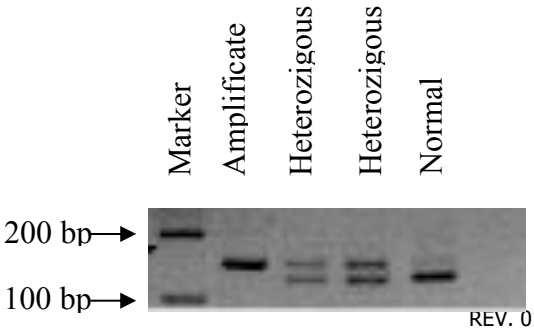
KIT CONTAINS AND STORAGE

Stability: over 18 months if correctly stored

ANALYSIS OF RESULTS

The yield of amplification is a fragment of 147 bp. The next restriction section made by the HpyCH4-IV enzyme gives the following results:

1 WILD TYPE subject	2 Heterozigous subject	3 Homozigous subject
2 bands **126 bp** **21 bp**	3 bands **147 bp** **126 bp** **21 bp**	1 band **147 bp**



References:
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