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KIT FOR THE DETECTION OF MUTATIONS: 5445DEL-4, 6696DELTC AND 9189DEL-4 IN BRCA2 GENE

AMPLI-set-BRCA2 Cat. n. 1.416

Breast cancer is the most frequent cancer type among women in the world, affecting up to 12% of all women in Europe and North America. The disease is usually sporadic, but in some cases it occurs in the presence of germinal mutations in predisposing genes. Two major genes associated with susceptibility to breast and ovarian cancer have been identified to date: BRCA1 and BRCA2 (Breast Cancer 1 and 2). The BRCA1 gene is on chromosome 17q12-21 and encodes a nuclear polypeptide of 220 KDa (1863 amino acids). BRCA1 has been implicated in several cellular functions, including repair of DNA damage, regulation of transcription, cell-cycle control. BRCA2 gene is located on chromosome 13q12.1 and it encodes a 384 Kda (3418 amino acids) Both the proteins are involved in many cell function as recombination and DNA repair, the regulation of cell cycle and of transcription. Germinal mutations in either of these genes increase the lifetime risk of developing breast and ovarian cancers. Hundreds of mutations, most of which are unique, have been identified throughout the entire coding sequences of both the BRCA1 and BRCA2 in different European and American populations, and they are uniformly located along the entire sequence of the gene.. More than 90% of mutations are frame shift or nonsense abnormalities, although single amino acid substitutions also arise. Ampli-set BRCA2 allows to detect, using the Polymerase Chain Reaction (PCR) , the mutations: 5445del-4, 6696delTC and 9189del-4. The detection is performed employing first a PCR reaction with specific primers pairs, followed by restriction cut by DraI (5445del-4),MnlI (6696delTC) and RsaI (9189del-4). The presence of mutation is confirmed by the loss of a restriction site.

Principle of assay: DNA extraction from whole blood, PCR with specific primers, Enzymatic digestion, Detection on agarose gel.
Applicability: On extracted and purified DNA from whole blood.
Test n: 24.

REAGENTS AND STORAGE

AMPLIFICATION	
PCR Mix 5445del-4	-20°C
PCR Mix 6696delTC	-20°C
PCR Mix 9189del-4	-20°C
H_2O RNase/DNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
DNA Normal Control	-20°C
DIGESTION	
Enzyme Dra I (20U/µl)	-20°C
Enzyme Mnl I (5U/µl)	-20°C
Enzima Rsa I (10U/µl)	-20°C
Buffer 10X Dra I	-20°C
Buffer 10X Mnl I	-20°C
Buffer 10X Rsa I	-20°C
BSA 100X	-20°C
H_2O RNase/DNase-free	-20°C

Stability: over 12 months if correctly stored.

ANALYSIS OF RESULTS

			Fragments obtained by enzymatic digestion	
Mix PCR	**P PCR product bp**	**Restriction enzyme**	**Normal subject**	**Presence of mutation**
5445del-4	**127 (123)**	**Dra I**	**98 29**	**123 98 29**
6696delTC	**131 (129)**	**Mnl I**	**95 36**	**129 95 36**
9189del-4	**146 (142)**	**Rsa I**	**127 19**	**142 127 19**

(-)In parenthesis is reported the PCR product of the mutated allele

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