ampli set BRCA1-2 30 tests (120 reactions) cat 1414

detection of mutations: 185delAG,188del-11bp and 5382insC in BRCA1 gene, and 6174delT in BRCA2 gene

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, and ubiquitination. 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. The ampli set BRCA1-2 kit allows the detection of the mutations, 185delAG, 188del-11bp e 5382insC of BRCA1 gene, and 6174delT of BRCA2. gene, using the polymerase chain reaction (PCR) with allele-specific oligonucleotide primers. Particularly, Mix A allows the simultaneous detection of three mutations: 185delAG, 5382insC and 617delT, whereas Mix B of 188del-11bp.

Principle of assay

DNA extraction from: whole blood
PCR with specific primers
Detection on agarose gel

Applicability

On extracted and purified DNA from whole blood

Analysis of results

Mix PCR A allows the simultaneous detection of three mutations (185delAG, 5382 insC e 6174delT), whereas **Mix PCR B** allows the detection of the 188del-11bp mutation. If the mutation is absent, only one PCR product is obtained with a primer pair. If the mutation is present on one of the alleles, two PCR product are produced with a primer pair.(see table below)

		PCR product (bp)	
	Allele-specific Primer	**Absence of mutation**	**Presence of mutation**
Mix PCR A	BRCA1 185delAG	**335**	**354** **335**
	BRCA1 5328insC	**271**	**295** **271**
	BRCA2 6174delT	**151**	**171** **151**
Mix PCR B	BRCA1 188del-11bp	**112**	**112** **101**

References

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