ampli set BRCA1 5 x 20 tests (100 reaction) cat 1415

detection of mutations:1499insA, 3596del4,4172insT,5083del19 and 5677insA in BRCA1 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. 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 kit allows the detection of the mutations , 1499insA, 3596del-4, 4172insT, 5083del-19 e 5677insA of BRCA1 gene , using the polymerase chain reaction (PCR) with allele-specific oligonucleotide primers. Particularly, the detection of 1499insA, 3596del-4,4172insT and 5677insA employs PCR reaction with specific primers pairs followed by restriction cut made by Ssp I(1499insA), BseGI (396del-4, 4172insT) and Rsa I(5677insA). The mutation is confirmed by loss of a cleavage site. The detection of mutation 5083del-19 is performed with PCR reaction because the presence of mutation is confirmed by the presence of a PCR product smaller of 19 bp.

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

Analysis of results

Product of PCR of normal subject is digested in fragments as shown in column “normal subject” in the table below. The presence of the mutation is confirmed by the loss of a restriction site and the presence of a fragment written in red in the column” presence of mutation”. It is suggested to load for every digestion reaction an undigested PCR product.
The detection of mutation 5083del-19 is performed with only a PCR reaction . The mutation is confirmed by a PCR product smaller of 19 bp than the normal DNA subject.

			Fragments obtained by enzymatic digestion	
Mix PCR	**PCR product bp**	**Restriction enzyme**	**Normal subject**	**Presence of mutation**
1499insA	**127 (128)**	**SspI**	**93** **34**	**128** **93** **34**
3596del4	**105 (101)**	**BseGI**	**86** **19**	**101** **86** **19**
4172insT	**109 (110)**	**BseGI**	**89** **20**	**110** **89** **20**
5677insA	**114 (115)**	**RsaI**	**53** **44** **17**	**70** **53** **44** **17**

Mix PCR	**PCR product bp**	**Normal subject**	**Presence of mutation**
5083del-19	**119 (100)**	**119**	**119** **100**

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

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