



ampli set BRCA2 3 x 20 tests (60 reaction) cat 1416

detection of mutations :5445del-4,6696delTC and 9189del-4 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. 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),MnII (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

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.

Mix PCR	PCR product bp	Restriction enzyme	Fragments obtained by enzymatic digestion	
			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

References

Miki Y. et al. (1994) Science 266:66-71

Wooster R. et al. (1995) Nature 378: 789-792

Ottini L. et al. ((2000) Human Mutation 431

Baudi F. et al. (2001) Breast Cancer Res 2: 307-310

Venkitaraman A.R.(2002) Cell 108: 171-182

Brose M.S. et al. (2002) J Natl Cancer Inst 94: 1365-72

Thompson D. et al. (2002) J Natl Cancer Inst 94: 1358-65

Mincey B.A. (2003) The Oncologist 8: 466-473.

Guttmacher, A.E. et al (2003) N Engl J Med 348: 2339-47

Stuppia L. et al. (2003) Human Mutation 635