Pineta Grande Hospital
Castel Volturno

dia-chem s.r.l.
DIAGNOSTICI E TECNOLOGIE BIOMEDICHE
BIOLOGIA MOLECOLARE

ACCREDIA
IQNet CISQ
UNI EN ISO 9001
UNI CEI EN ISO 13485

CE

KIT FOR THE DETECTION OF MUTATIONS: 1499insA, 3596DEL4,4172insT,5083del19 AND 5677insA IN BRCA1 GENE

AMPLI-SET BRCA1 Cat. n. 1.415

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 frameshift or nonsense abnormalities, although single aminoacid substitutions also arise. The AMPLI-SET BRCA1kit 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.

Test: 24

REAGENTS AND STORAGE

AMPLIFICATION	
Mix PCR 1499insA	-20°C
Mix PCR 3596del-4	-20°C
Mix PCR 4172insT	-20°C
Mix PCR 5677insA	-20°C
Mix PCR 5083del-19	-20°C
H_2O RNase/DNase-free	-20°C
Taq Polymerase (5U/µl)	-20°C
Normal DNA control	-20°C
DIGESTION	
Ssp I Enziyme (5U/µl)	-20°C
BseGI Enziyme (10U/µl)	-20°C
Rsa I Enziyme (10U/µl)	-20°C
Buffer 10X Ssp I	-20°C
Buffer 10X BseGI	-20°C
Buffer 10X Rsa I	-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	**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**

(-) 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. (2202) 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