KIT FOR THE DETECTION OF METHYLATION OF PROMOTER OF GENES INVOLVED IN BREAST CANCER

AMPLI-SET-Breast Cancer Cat. n.1.412

The methylation of the residues of cytosine in the "CpG islands" is very important for the regulation of the genic expression. The hyper- methylation of the "CpG islands" in the promoter region of a gene suppress the transcription of the same gene .In many tumours the hypermethylation of the promoter of the suppressor genes, as p16, p15, E-cadherine and other genes as "DAP-kinase", inhibitor gene of the metastatic progression , 06-metilguanina DNA methyltransferase (MGMT), gene involved in the repair of DNA, Glutathione-S-transferase (GSPT1) involved in the prevention of the oxidative damage of DNA etc.
Plasma and serum of patients carrier of malignant neoplasia contains much genomic DNA than the control subjects.
The principle of the assay is the extraction of genomic DNA from plasma or serum , the treatment with bisulfite sodium in order to transform the unmethylated residue of cytosine in uracil, the PCR amplification with specific oligonucleotides for the methylated sequences and unmethylated (MSP:methylation specific PCR) followed by the detection by electrophoresis on agarose gel. The assessment of the state of hypermethylation of a gene is an appreciable molecular marker of the risk, and allows a precocious diagnosis and a prognosis of a neoplastic diseases .The kit allows the detection of methylation of the promoter of the onchosuppressor p16 , of the BRCA1 gene, involved in the repair of DNA, and of the glutathione-S- transferase (GSTPI), which show respectively a hypermethylation rate of the 15-25%, of the 30% and of the 30/60% in non-hereditary breast cancer.

Principle of Assay: A) extraction of genomic DNA B) Modification treatment with sodium bisulfite C)amplification with specific primers for methylated and unmethylated sequences of the promoter of the genes: BRCA1 – p16 – GSTP1, D) detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood sample

MODIFICATION	
NaOH	-20°C
Reagent A (sodium bisulfite)	-20°C
Raegent B (idrochinone)	-20°C
Reagent C (resin)	-20°C
Reagente (carrier)	-20°C
Diluente	-20°C
Sterile H_2O	-20°C
AMPLIFICATION	
Mix PCR **METHYLATED BRCA1**	-20°C
Mix PCR UNMETHYLATED **BRCA1**	-20°C
Mix PCR **METHYLATED p16**	-20°C
Mix PCR UNMETHYLATED p16	-20°C
Mix PCR **METHYLATED GSTP1**	-20°C
Mix PCR UNMETHYLATED GSTP1	-20°C
sterile H_2O	-20°C
Taq Polymerase (5U/□l)	-20°C
Unmethylated DNA Control	-20°C
Methylated DNA Control	-20°C

Numbers of Tests: 24
Stability: over 12 months if correctly stored.

References:
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3)Esteller M. et al Oncogene 16, 2413-2417 (1998)
4)Esteller M. et al Cancer Res 59, 67-70 (1999)
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6)Leon S. A. et al. Cancer Res 37, 646-650 (1977)
7)Stroun M. et al Oncology 46, 318-322 (1989)
8)Shapiro B. et al Cancer 51, 2116-2120 (1983)
9)Wong I. H. N. et al Cancer Res 59, 71-73 (1999)
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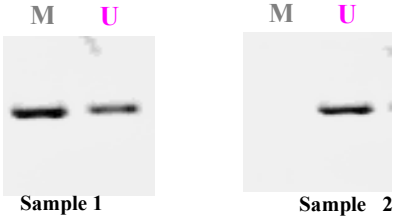
ANALYSIS OF RESULTS

Every sample of DNA, after treatment with sodium bisulfite, has been amplified both with METHYLATED (M) mix and UNMETHYLATED (UM) mix using specific oligonucleotides included in the kit for the methylated and unmethylated sequences of the p16, GSTP1 e BRCA1 genes
Every PCR product must be run on agarose gel separately (it is suggest to put the product of amplification of every sample near each other on the gel). Samples where a methylated state of the promotor of the gene is present , give a band both with the METHYLATED mix and the UNMETHYLATED mix.
Samples where an methylated state of the promotor of the gene isn't present, give a band only with the UNMETHYLATED mix. Specifically, the analysis of the status of methylation of the promotor of the P16 gene gives a band of 150 bp with the METHYLATED MIX and a band of 151 bp with UNMETHYLATED mix . The analysis of the status of methylation of the promotor of the GSTP1 gene gives a band of 91 bp with the METHYLATED mix and a band of 97-bp with the UNMETHYLATED mix. Finally, the analysis of the status of methylation of the promotor of the BRCA1 gene gives a band of 182-bp both with the METHYLATED mix and the UNMETHYLATED mix.

Example: analysis of methylation status of GSTP1 gene



Sample 1
PRESENCE OF METHYLATED STATUS OF THE GSTP1

Sample 2
ABSENCE OF METHYLATED STATUS OF THE GSTP1

N.B. The DNA unmethylated Control , after treatment with sodium bisulfite, wiil show a pCR product only with the Unmethylated Mix