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KIT FOR THE DETECTION OF THE HYPERMETHYLATION STATE OF PROMOTER OF GENE GLUTATHIONE-S-TRANSFERASE (GSTP1) IN PROSTATE CANCER

AMPLI-SET-GSTP1 Cat. n. 1.410

The methylation of the residues of cytosine in the "CpG islands" is very important for the regulation of genic expression. The hypermethylation 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, O^6 –methylguanina DNA methyltransferase (MGMT), gene involved in the repair of DNA, Glutathione-S-transferase (GSTP1) involved in the prevention of the oxidative damage of DNA, etc has been showed.
Hypermethylation of "CpG islands", moreover, represents an useful therapeutic "target": the restoration of "silenced genes" might be possible via treatment with inhibitors of CpG methylation . The detection of the hypermethylation state of a gene can be an useful molecular biomarker for screening, early diagnosis and follow-up of neoplastic diseases.
The inactivation due to the hypermethylation of the gene encoding for the Gluthatione-S-transferase (GSTP1) is a "bio-marker" for the human prostate cancer (PCA) Tumour cells contain CpG hyperrmethylated sequences in the regulatory region of the promoter Because of the "gene-silencing" the level of the protein produced by the cells is very low These epigenetic changes occur very early in the development of the tumour, and the cells become vulnerable to oxidants and electrophiles. The kit allows the detection of the methylation of the promoter of the GSTP1 gene.
The principle of the assay is the extraction of genomic DNA from serum, or plasma or tissue, the treatment with bisulphite sodium in order to convert the unmethylate residue of cytosine in uracil, the PCR amplification with specific oligonucleotides for the methylate sequences and unmethylated sequences (MSP: methylation specific PCR) followed by the detection by electrophoresis on agarose gel.

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 GSTP1 D) detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood samples.
Numbers of Tests: 24

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

REAGENTS and STORAGE

Stability: over 12 months if correctly stored.

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 GSTP1 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. The 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.

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, will show a pCR product only with the Unmethylated Mix

References:
1) Cancer Epidemiol Biomarkers Prev 2002 May; 11 (5): 445-50.
2) Oncogene (2002) 21, 1048-1061.
3) Oncogene (1999) 18, 1313-1324.
4) J. Biol. Chem. (2000) 275, 24893-24899.