METHYLATION STATUS OF GSTP1 GENE PROMOTOR DETECTION BY REAL TIME PCR

AMPLI-SET-GSTP1 RT　　Cat. n. 1.410RT

The methylation of the residues of cytosine in the "CpG islands" is very important for the regulation of the 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 –metilguanina DNA metiltransferase (MGMT), gene involved in the repair of DNA, Glutatione-S-transferasi (GSTP1) involved in the prevention of the oxidative damage of DNA, etc has been showed.

The detection of the gene promoter methylation can be performed on genomic DNA. Plasma and serum of patients carrier of malignant neoplasia contains much genomic DNA than the control subjects (up to 4 times as much) The assessment of the state of hyper-methylation of a gene is an appreciable molecular marker of the risk, and allows a precocious diagnosis and a prognosis of neoplastic diseases.

The inactivation due to the hypermethylation of the gene encoding for the Glutatione-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 using the Real Time PCR technique.

Principle of assay : A) isolation of genomic DNA B) bisulfite modification C)Real-Time PCR
Applicability: on DNA isolated from samples of serum/plasma or fresh/paraffin-embedded specimen
Number of Test: 24.

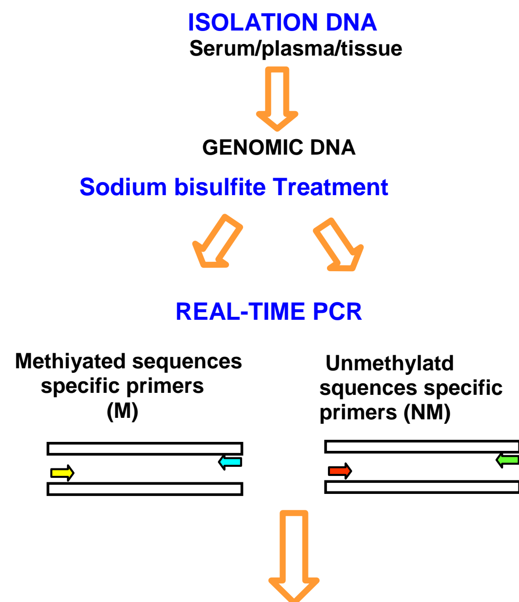
Kit contents and storage

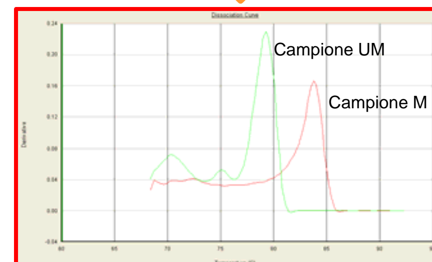
AMPLIFICATION	storage
Mix PCR METHYLATED	-20°C
Mix PCR UNMETHYLATED	-20°C
H_2O Sterile	-20°C
Taq Polymerase (5U/µl)	-20°C
Unmethylated DNA control	-20°C
Methylated DNA control	-20°C

Stability: over 18 months if correctly stored
Required material: 1,5 ml tubes refreshed tube tray filter sterile tips; 96 wells microplate

Required equipment:
Pre- and post PCR pipettes set
1) 0.5 – 10 microliters
2) 5 – 50 microliters
3) 50 – 200 microliters
Class II biolocigal safety cabinet Real-Time PCR Instrument.

PRINCIPLE OF ASSAY





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