

METHYLATION STATUS OF hMLH1 GENE PROMOTOR DETECTION BY REAL TIME PCR

AMPLI-SET-hMLH1 RT

Cat. n. 1.420RT

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, O⁶-methylguanine 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.

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.

Tumour cells contain CpG hypermethylated 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 hMLH1 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 unmethylated residue of cytosine in uracil, the PCR amplification with specific oligonucleotides for the methylated 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.

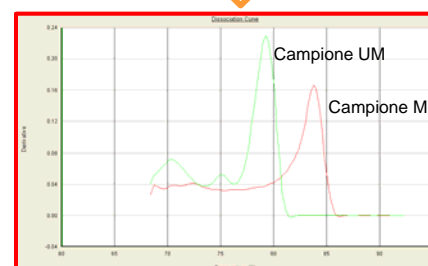
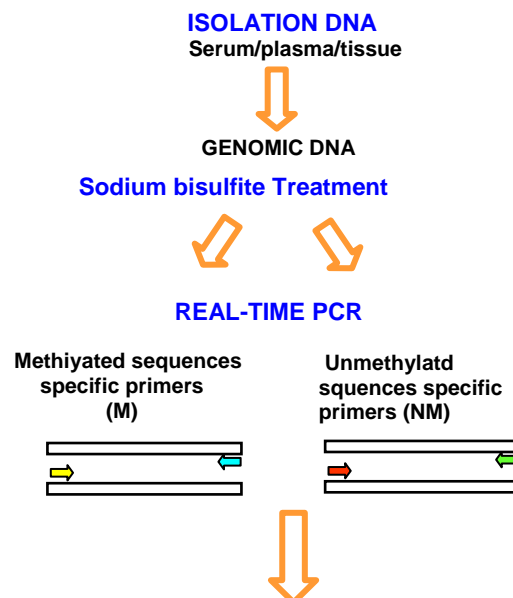
KIT CONTENTS AND STORAGE

AMPLIFICATION

Mix PCR METHYLATED	-20°C
Mix PCR UNMETHYLATED	-20°C
H ₂ O 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.

ANALYSIS OF RESULTS



References:

- 1) Sanchez-Cespedes M. et al *Cancer Res* 60, 892-895 (2000)
- 2) Herman J. G. et al *Proc. Natl. Acad. Sci. USA* 95, 6870-6875 (1998)
- 3) Esteller M. et al *Oncogene* 16, 2413-2417 (1998)
- 4) Esteller M. et al *Cancer Res* 59, 67-70 (1999)
- 5) Esteller M. et al *Cancer Res* 59, 793-797 (1999)
- 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)
- 10) Baylin S. B. *Adv. Cancer Res.* 72, 141-196 (1998)
- 11) Belinsky S. A. *Proc. Natl. Acad. Sci. USA* 95, 11891-11896 (1998)
- 12) *Int J Cancer*: 102, 623-628 (2002).