



BIOLOGIA MOLECOLARE



ASESSMENT OF GENE MGMT METHYLATION STATE OF p16 PROMOTER GENE IN REAL TIME AMPLI-SET-p16 RT Cat. n. 1.417RT

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—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 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 kit allows the detection of the methylation state of the promoter of the p16 gene.

The detection of the gene promoter methylation can be performed on genomic DNA from tissue, flaking cells or from serum of patients carrier of malignant neoplasia contains much genomic DNA than the control subjects (up to 4 times as much).

The principle of the assay is the extraction of genomic DNA, 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).

Principle of Assay: A) extraction of genomic DNA B) Modification treatment with sodium bisulfite C)amplification and revelation by real-time PCR

Applicability: On extracted and purified genomic DNA from serum/plasma or fresh/paraffin embedded tissue.

Numbers of Tests: 24

REAGENTS AND STORAGE

<u>AMPLIFICATION</u>	CONSERVATION
Mix PCR METHYLATED	-20°C
Mix PCR UNMETHYLATED	-20°C
H ₂ O Sterile	-20°C
Taq Polymerase (5U/μl)	-20°C
Un-methylated Control DNA non metilato	-20°C
Methylated control DNA	-20°C

Stability: over 18 months if correctly stored.

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)Belinsky S. A. Proc. Natl. Acad. Sci. USA 95, 11891-11896

PRINCIPLE OF METHOD

DNA EXTRACTION Serum/plasma/tissue



TREATMENT con Sodium Bisulfite





REAL-TIME PCR

