DETECTION OF METHYLATION OF PROMOTER OF GENES INVOLVED IN LUNG CANCER

AMPLI-SET Lung Cancer Cat. n.1.411

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 tumors the hyper- methylation of the promoter of the supprressor genes, as p16, p15, E-cadherine and other genes as "DAP-kinase", inhibitor gene of the metastatic progression , 06-metilguanina DNA metiltransferase (MGMT), gene involved in the repair of DNA, Glutatione-S-transferasi (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 convert the unmethylate 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 the methylation of the promoter of the tumour suppressor gene p16 , of the DAP-kinase gene and of the 06-methylguanine DNA methyltransferase (MGMT). In the carcinoma of the lung 68% of the patients show at least one of these genes hyper-methylated.

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: p16 - DAP-Kinase – MGMT, D) detection on agarose gel.
Applicability: On extracted and purified genomic DNA from whole blood samples.
Numbers of Tests: 24

REAGENTS and STORAGE

Stability: over 12 months if correctly stored.

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 MGMT**	-20°C
Mix PCR UNMETHYLATED **MGMT**	-20°C
Mix PCR **METHYLATED p16**	-20°C
Mix PCR UNMETHYLATED p16	-20°C
Mix PCR **METHYLATED DAP-kinase**	-20°C
Mix PCR UNMETHYLATED DAP-kinase	-20°C
sterile H_2O	-20°C
Taq Polymerase (5U/□l)	-20°C
Unmethylated DNA Control	-20°C
Methylated DNA Control	-20°C

References

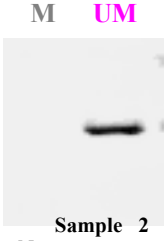
1)Sanchez-Cespedes M. et al Cancer Res 60, 892-895 (2000)
2)Herman J. G. et al Proc. Natl. Acad. Sci. USA 95, 6870-6875 (1998)
3)Esteller M. et al Oncogene 16, 2413-2417 (1998)
4)Esteller M. et al Cancer Res 59, 67-70 (1999)
5)Esteller M. et al Cancer Res 59, 793-797 (1999)
6)Leon S. A. et al. Cancer 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).

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, MGMT and dap-KINASE 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 theUNMETHYLATED 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 promoter of MGMT gene gives a band of 98 bp with METHYLATED mix and of 106 bp with the UNMETHYLATED mix PCR. The analysis of the status of methylation of the promoter of the DAP-Kinase gene gives a band of 98 bp with the METHYLATED mix PCR and a band of 108 bp with the UNMETHYLATED.
The same result will be obtained after a II PCR

Example: analysis of methylation status of p16 gene

Sample 1
POSITIVE FOR THE METHYLATION OF PROMOTER P16



Sample 2
NEGATIVE FOR THE METHYLATION OF PROMOTER OF P16

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