

# MGMT gene promoter methylation (O<sup>6</sup>-methylguanine DNA methyltransferase)

## ORDERING INFORMATIONS

REF: *ONC-001-25*  
 CND Code: *W01060299*  
 RDM Code: *2256631/R*  
 Tests: *25 Reactions: 31 x 2*  
 Manufacturer: *BioMol Laboratories s.r.l.*

## CONTENTS OF THE KIT

*The kit consists of: reagents for modification with sodium bisulfite and for amplification in MSP-PCR \*reagents for the extraction of genomic DNA are not supplied in the kit.*

For in vitro diagnostic use



## PRODUCT CHARACTERISTICS

Device belonging to the family of in vitro medical devices **REAL-TIME QUALITATIVE PCR IN EPIGENETICS**. Determination of the methylation status of the promoter of the MGMT (O<sup>6</sup>-methylguanine DNA methyltransferase) gene by MSP (methylation-specific PCR) technique and subsequent detection by Real-Time PCR technique. Kit optimized for Biorad CFX96 Dx, Biorad Opus Dx and Agilent AriaDx real-time PCR instrumentation.

## SCIENTIFIC BACKGROUND

DNA O<sup>6</sup>-methylguanine methyltransferase (MGMT) is a DNA repair enzyme encoded by the MGMT gene present at the 10q26 locus. The MGMT enzyme removes the alkyl groups from the O<sup>6</sup> position of guanine acting itself as an acceptor and this reaction leads to an irreversible inactivation of the enzyme. MGMT transcription is regulated by epigenetic mechanisms. Indeed, methylation of CpG dinucleotides in the promoter region of MGMT causes gene silencing, loss of MGMT expression and inability to remove alkyl groups from methylated guanine with consequent alteration of the normal DNA structure.

*§ Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 1996 Sep 3; 93(18): 9821-9826.*

*§ Role of MGMT as biomarker in colorectal cancer. World J Clin Cases 2014 Dec 16; 2(12): 835-839.*

*§ Characterizing DNA methylation alterations from The Cancer Genome Atlas. J Clin Invest 2014 Jan 2; 124(1): 17-23.*

*§ Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999 59: 67-70.*

## CLINICAL SIGNIFICANCE

MGMT protects normal cells from carcinogens, but the activity of MGMT also protects cancer cells from the lethal effects of chemotherapy with alkylating agents such as dacarbazine (DTIC) or temozolomide (TMZ), which are widely used for the treatment of melanoma and glioblastoma. In fact, MGMT removes the methyl groups from the O<sup>6</sup> position of the guanines, thus making TMZ ineffective.

In glioblastomas, MGMT promoter methylation is predictive of the therapeutic benefit of the alkylating agent temozolomide, as shown in several phase III clinical trials, and MGMT gene methylation status has become the first predictive biomarker in neuro-oncology.

MGMT gene promoter methylation also plays an important role in colorectal carcinogenesis, occurring in approximately 30%-40% of metastatic colorectal cancer. Its prognostic role is not yet defined, but the loss of MGMT expression, which is secondary to gene promoter methylation, results in an interestingly high response to alkylating agents.

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DESCRIPTION	LABEL	VOLUME	STORAGE
		<b>ONC-001-25</b>	
Conversion reagent	Conversion reagent	3 tubes	RT
Dilution buffer	Buffer A	900 µl	RT
Buffer	Buffer B	200 µl	RT
Buffer	Buffer C	15 ml	RT
Wash buffer	Buffer D	3 ml	RT
Buffer	Buffer E	5 ml	RT
Buffer	Buffer F	750 µl	RT
Columns	Columns	25	RT
Collection tubes	Collection tubes	25	RT
Mix oligonucleotides	Mix A methylated 10X	1 x 85 µl	-20°C
Mix oligonucleotides	Mix B unmethylated 10X	1 x 85 µl	-20°C
Mix buffer and Taq polymerase enzyme	Mix Real time PCR 2X	1 x 850 µl	-20°C
Deionized H <sub>2</sub> O	Deionized H <sub>2</sub> O	2 x 1 ml	-20°C
Genomic DNA or recombinant DNA Methylated and unmethylated	Control +	100 µl	-20°C

## TECHNICAL CHARACTERISTICS

### COD. ONC-001-25

STABILITY	18 months
REAGENTS STATUS	Ready to use
BIOLOGICAL MATRIX	Genomic DNA extracted from whole blood, tissue, cells
POSITIVE CONTROL	Recombinant dna for at least 4 analytical session
TECHNOLOGY	Real-time PCR; oligonucleotides and specific probes; 1 SYBR-GREEN/FAM fluorescence channel
VALIDATED INSTRUMENTS	Biorad CFX96 Dx, Biorad Opus Dx e Agilent AriaDx
RUNNING TIME	150 min
THERMAL CYCLING PROFILE	1 cycle at 95 °C (15 min); 45 cycles 95 °C (40 sec) +60 °C (40 sec) + 72 °C (40 sec); 1 dissociation cycle at 70 °C with an increase of 0,2 °C
ANALYTICAL SPECIFICITY	Absence of non-specific pairings of oligonucleotides and probes; absence of cross-reactivity
ANALYTICAL SENSITIVITY : LIMIT OF DETECTION (LOD)	≥ 2.5 ng of sodium bisulfite modified DNA
ANALYTICAL SENSITIVITY : LIMIT OF BLANK (LOB)	0% NCN
REPRODUCIBILITY	99,9%
DIAGNOSTIC SPECIFICITY / DIAGNOSTIC SENSITIVITY	100%/98%