DIACARTA®

Brain Tumor

MGMT Gene Promoter Methylation Detection Test

High-grade gliomas or primary malignant glioblastoma

MGMT promoter as a strong and independent prognosis biomarker

Methylation of the MGMT (O6 -methylguanine-DNA methyltransferase) gene promoter reduces the amount of the MGMT enzyme and decreases the DNA repair capability in cancer cells. Therefore, cancer cells are more responsive to radiation or temozolomide (TMZ) chemotherapy that damages DNA and the patients with methylation of MGMT promoter show better prognosis, especially for stage IV glioma patients.

DiaCarta has developed the QMethyl™ MGMT Gene Promoter Methylation Test for research use and validation of clinical labs if the biomarker is desired for clinical decisions.

Introducing QMethyl™ MGMT Gene Promoter Methylation Test

The MGMT methylation assay is a high-throughput quantitative approach that utilizes fluorescence-based real-time PCR (TaqMan) technology to identify methylated and unmethylated DNA. This methylation assay contains PCR primers with no CpG sites so that the status of CpG methylation would not affect the primer binding. The assay comprises dual-labeled TaqMan probes for 5 CpG sites complementary to either the methylated or unmethylated sequence. One FAM-labeled probe recognizes the methylated sites, and VIC labeled probe recognizes the unmethylated sites. If the sequence exhibits high methylation or unmethylation, it will bind the complementary probe, and fluorescence is generated quantitatively **(Figure 1)**. The methylation status of a sample was determined by the Cq (quantification cycle) value.

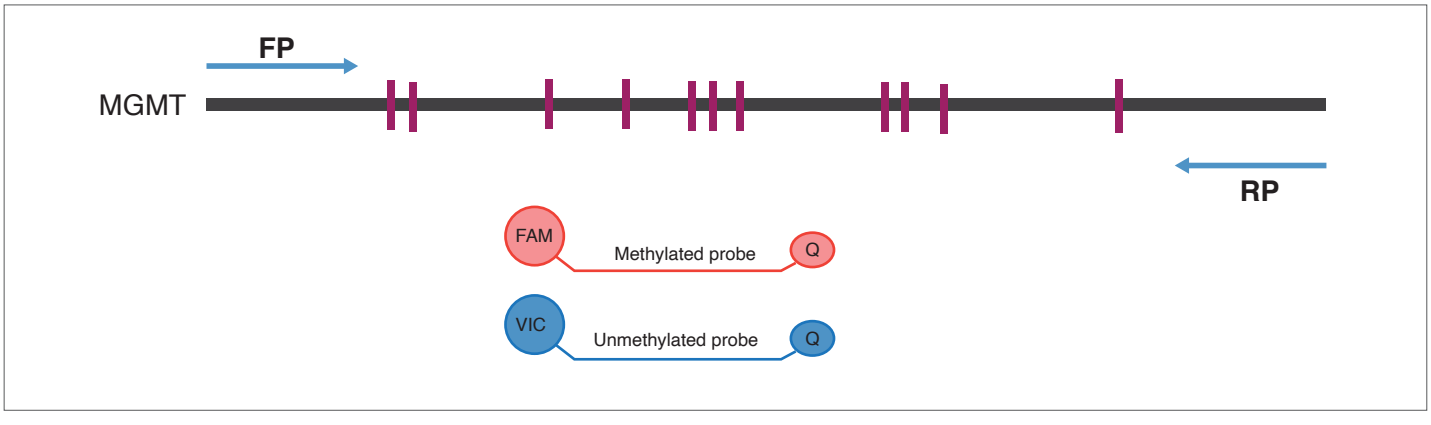

Figure 1: Schematic representation of MGMT qPCR methylation assay. The CpG is represented as red vertical lines. The blue arrows represent forward and reverse primers. The probes are labeled with two different fluorochromes to identify methylated and unmethylated DNA. FP- Forward primer, RP- Reverse Primer, FAM or VIC- Fluorescein amidites, Q-Quencher.

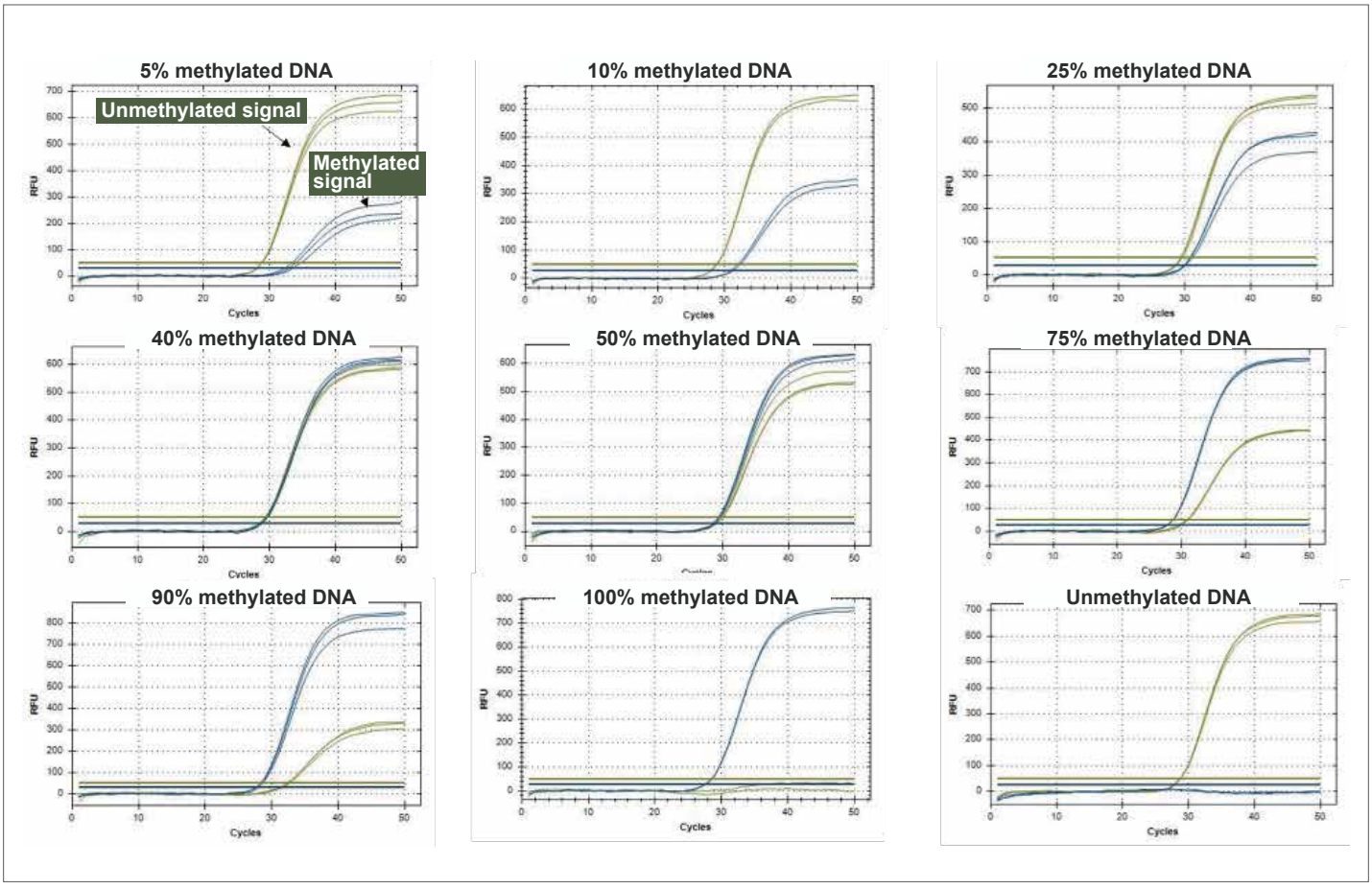

Figure. 2: The MGMT amplification plots for spike-in samples (5% to 90% methylated DNA), PC and NC. The methylation-specific TaqMan probe (blue) contains FAM, whereas the unmethylation specific TaqMan probe (green) contains VIC. The methylation rate was calculated using the threshold cycle (CT) of the FAM channel (CG reporter, blue) and the VIC channel (TG reporter, green).

Analytical performance of the test

The analytical accuracy was verified and validated by testing well-characterized samples with known methylation verified by NGS bisulfite sequencing. The results demonstrated a 100% match between reference methods and the MGMT qPCR methylation kit.

Table 1: Assay reproducibility and precision

Variation-Methylated probe	Methylated probe (% CV)	Unmethylated probe (% CV)
Inter-assay	≤7%	≤2%
Intra-assay	≤11%	≤1%
Instrument Variability	≤11%	≤1%
Operator Variability	<10%	<3%

Table 2: Assay reproducibility and precision

Spike-in methylated DNA (%) 20 replicates	ABI 7500Dx	
	Methylated probe (% correct call)	Unmethylated probe (% correct call)
0.5%	10%	100%
1%	70%	100%
2%	100%	100%
4%	100%	100%
100% methylated DNA (PC)	100%	100%
0% methylated DNA (NC)	100%	100%

Clinical performance of the test

Table 3: Summary of MGMT gene methylation qPCR test for glioblastoma FFPE samples

Pathological stages	MGMT qPCR methylation test	Clinical status		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Stage II	Positive	1	0	33% (95%CI-17.6-87.4)	100% (95%CI:19.7-100.0)
	Negative	2	2		
	Total	**3**	**2**		
Stage III	Positive	4	0	100% (95%CI:39.57-100.0)	100% (95%CI:19.7-100.0)
	Negative	0	2		
	Total	**4**	**2**		
All stages	Positive	5	0	71.4% (95%CI: 30.2-94.8)	100% (95%CI:19.7-100.0)
	Negative	2	2		
	Total	**7**	**2**		

Product Name: QMethyl™ MGMT Gene Promoter Methylation Test
Pack size: 24 reactions
Catalog Number: DC-14-0001R
For research use