Crystal Digital PCR® Assay

Information Sheet

For Research Use Only. Not for use in diagnostic procedures.

Product Name

MGMT Methylation Crystal Digital PCR® Assay (R51053)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
MGMT Methylated/Unmethylated Promoter	DNA	Blue/Green	2

The MGMT Methylation Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify methylation in the promoter of the MGMT gene using the Ruby Chip. MGMT plays critical roles in DNA repair mechanisms: it is involved in the direct reversal of DNA alkylation damage. Promoter hypermethylation is the most common mechanism of gene silencing, leading to loss of function. This epigenetic inactivation contributes significantly to genomic instability and uncontrolled cell proliferation in a variety of cancers.

Multiplexing Strategy: Color-Combination

This assay relies on the Color-Combination multiplexing strategy proprietary to Stilla Technologies, in which targets are detected, differentiated, and quantified by Crystal Digital PCR® using 2 fluorophores.

The table below indicates with a “X” which channel(s) are used for each target in the assay:

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
MGMT Unmethylated DNA	X						
MGMT Methylated DNA			X				

Components

MGMT Methylation Crystal Digital PCR® Assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a Positive Control. Please refer to the lot specific Certificate of Conformity for characterized concentration, available for download at the Technical Resources section of the Stilla Technologies website.

Component Name	Reference	Concentration	Description
MGMT Methylation Crystal Digital PCR® Assay	R51053	10X	Detects methylation of the MGMT promoter
MGMT Positive Control	R51053.PC0	10X	Contains: synthetic sequences corresponding to methylated and unmethylated DNAs after bisulfite treatment

Specific Recommendation Regarding Sample Treatment and DNA Input

The assay is designed to detect methylated and non-methylated sequences after bisulfite treatment. Samples must therefore first be subjected to bisulfite treatment. The kit used during assay validations is indicated in section “Consumables Required but Not Provided”.

Thermocycling Programs

On the Nio® Digital PCR:

Step		Ramp rate
Step 1	Partition for Ruby Chip	-
Step 2	Temperature 95°C for 180 seconds	1°C/sec
Step 3	Begin Loop for 60 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	2°C/sec
Step 3.2	Temperature 60°C for 60 seconds	2°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

Image Acquisition

Download the dedicated scanning file from the Technical Resources section of the Stilla Technologies website:

- NioProtocol_3C-60X-60C-60s.nioprotocol
- NioAssay_3C_MGMT_R51053.nioassay

Image Analysis

The following files are embedded in the dedicated scanning files listed above:

- UniversalCompMatrix_3C_Prism6-Nio.ncm
- AnalysisConfiguration_MGMT_R51053.nca

Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 3 (R41401 200 reactions, R41402 1000 reactions)
- Nuclease-free water
- Bisulfite conversion kit (Example: EZ DNA Methylation-Lightning Kit, ref: ZD5030 or ZD5030-E from Ozyme)

Instruction for PCR Mix Preparation

Specific instructions for preparing the PCR mix are given below.

Reagent Name	Initial Concentration	Final Concentration	Volume per reaction (µL)
naica® PCR MIX Buffer A	10x	1x	0.60
naica® PCR MIX Buffer B	100%	4%	0.24
Crystal Digital PCR® Assay	10x	1x	0.60
Crystal Universal Reporter Tube A	40x	1x	0.15
Nuclease-free water	NA	NA	Variable
Template DNA	**NA**	**NA**	**Variable**
or Positive Control	*10x*	*1x*	*0.60*
		Total reaction volume (µL)	*6.0*

Representative Data and Instructions for Analysis

Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis, the Blue/Green thresholds should be set above the negative cluster. Examples of results obtained on the Nio™+ system are given below.

Remark: The threshold can be adjusted on each individual chamber to optimize its placement.

Wet lab testing was carried out using H_2O as a negative control and a positive control consisting of synthetic DNAs corresponding to methylated and unmethylated DNA sequences after bisulfite treatment. Synthetic DNAs, hgDNA and methylated DNA standard (CpG Methylated Human Genomic DNA, ref: SD1131 from ThermoFisher) were also tested individually after bisulfite treatment.

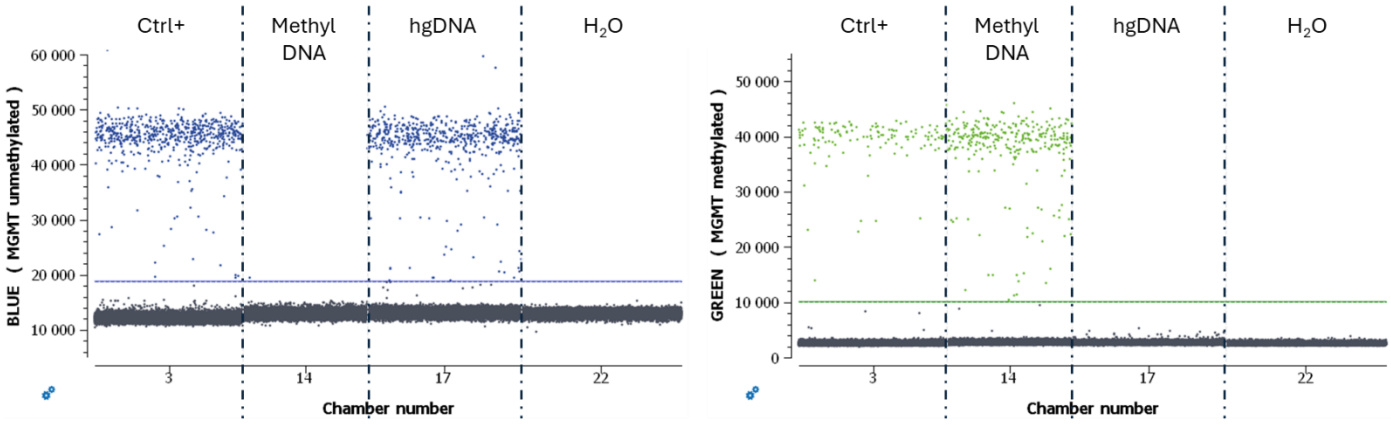

Figure 1: 1D plots obtained during wet lab testing on the Nio™+. *The thresholds should be set above negative cluster.*

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