

# Crystal Digital PCR® Assay

## Information Sheet

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

### Product Name

GSTP1 Methylation Crystal Digital PCR® (R51003)

### Description

#### Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
<b>GSTP1 Methylated/Unmethylated Promoter</b>	DNA	Green/Yellow/Red	2

The GSTP1 Methylation Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify methylation of the GSTP1 promoter using the Ruby Chip. The GSTP1 promoter is implicated on the GSTP1 epigenetic silencing and dysregulation of detoxification pathways contributing to tumor initiation, progression, and drug resistance.

#### Multiplexing Strategy: Color-Combination

This assay relies on the Color-Combination multiplexing strategy proprietary to Stilla Technologies, in which each target is 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
<b>GSTP1_Unmethylated-DNA</b>			X		X		
<b>GSTP1_Methylated-DNA</b>			X	X			

### Components

GSTP1 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
<b>GSTP1 Methylation Crystal Digital PCR® Assay</b>	R51003	10X	Detects methylation of the GSTP1 promoter.
<b>BRCA1/RAD51C/GSTP1 Positive Control</b>	R51008.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”.

To ensure optimal performance, it is recommended not to exceed a DNA concentration in the Ruby chamber of 300 cp/μL, which corresponds to 1 ng/μL or 6 ng in the 6 μL of PCR mix prepared.

## Thermocycling Programs

### On the naica® system:

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

### On the Nio™ Digital PCR:

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

## Image Acquisition

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

- ScanningTemplate\_Prism6\_GSTP1\_R51003.ncx (6-color naica® system)
- NioProtocol\_6C-60X-60°C-60s.nioprotocol (Nio™ Digital PCR)
- NioAssay\_6C\_GSTP1\_R51003.nioassay (Nio™ Digital PCR)

## Image Analysis

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







- CompensationMatrix\_Prism6\_GSTP1\_R51003.ncm (6-color naica® system)
- CompensationMatrix\_Nio\_GSTP1\_R51003.ncm (Nio™ Digital PCR)
- AnalysisConfiguration\_GSTP1\_R51003.nca (all systems)

## Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Universal Reporters 7 (R42401 200 reactions, R42402 1000 reactions)
- Nuclease-free water
- Bisulfite conversion kit (Example: EZ DNA Methylation-Gold Kit, ref: ZD5005 or ZD5006 from Ozyme)

## Instruction for PCR Mix Preparation

To ensure good assay performance, the final concentration of naica® PCR MIX Buffer B should be fixed at 2%. Specific instructions for preparing the PCR mix are given below.

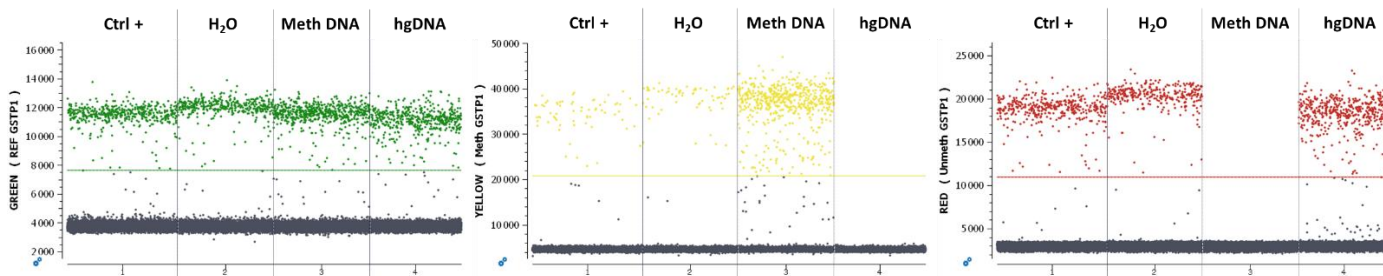
Reagent Name	Initial Concentration	Final Concentration	Volume per reaction (µL)
naica® PCR MIX Buffer A 	10x	1x	0.60
naica® PCR MIX Buffer B 	100%	2%	0.12
Crystal Digital PCR® Assay 	10x	1x	0.60
Crystal Universal Reporter Tube A 	40x	1x	0.15
Crystal Universal Reporter Tube B 	40x	1x	0.15
Nuclease-free water	NA	NA	Variable
<b>Template DNA</b>	<b>NA</b>	<b>NA</b>	<b>Variable</b>
<i>or Positive Control</i> 	10x	1x	0.60
<i>Total reaction volume (µL)</i>			<b>6.0</b>

## Representative Data and Instructions for Analysis

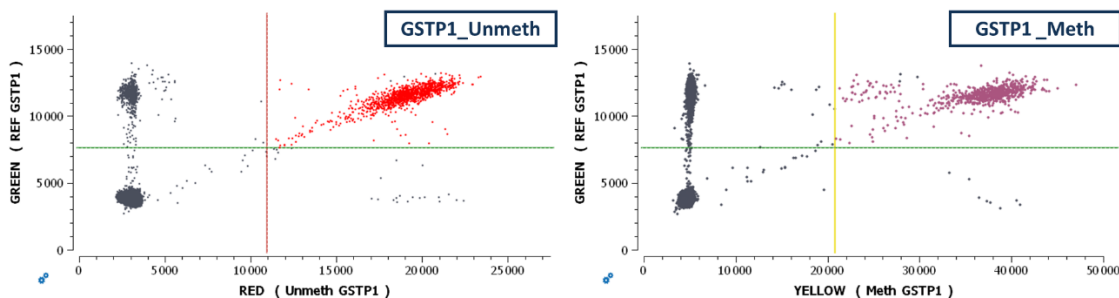
Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis, the Green/Yellow/Red thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the 6-color naica® system are given below.

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

Wet lab testing was carried out using H<sub>2</sub>O as a negative control and a positive control consisting of synthetic DNAs corresponding to methylated and unmethylated DNA sequences after bisulfite treatment. 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 6-color naica® system. The thresholds should be set at approximately equal distance from the positive and negative clusters.**



**Figure 2: 2D plots obtained during wet lab testing on the 6-color naica® system. The Green/Red double positive population corresponds to unmethylated DNA while the Green/Yellow double positive population corresponds to methylated DNA.**



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