Crystal Digital PCR® Assay

Information Sheet

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

Product Name

RAD51C Methylation Crystal Digital PCR® (R51005)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
RAD51C Methylated/Unmethylated Promoter	DNA	Green/Red/Infra-Red	2

The RAD51C Methylation Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify methylation of the RAD51C promoter using the Ruby Chip. RAD51C gene is involved in DNA repair, particularly in the homologous recombination pathway for genome stability.

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
RAD51C_Unmethylated-DNA					X	X	
RAD51C_Methylated-DNA			X			X	

Components

RAD51C 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
RAD51C Methylation Crystal Digital PCR® Assay	R51005	10X	Detects methylation of the RAD51C promoter.
BRCA1/RAD51C/GSTP1 Positive Control	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
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	1°C/sec
Step 3.2	Temperature 60°C for 60 seconds	1°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

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:

- ScanningTemplate_Prism6_RAD51C_R51005.ncx (6-color naica® system)
- NioProtocol_6C-60X-60°C-60s.nioprotocol (Nio™ Digital PCR)
- NioAssay_6C_RAD51C_R51005.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_RAD51C_R51005.ncm (6-color naica® system)
- CompensationMatrix_Nio_RAD51C_R51005.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_RAD51C_R51005.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
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 Green/Red/Infra-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_2O 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.

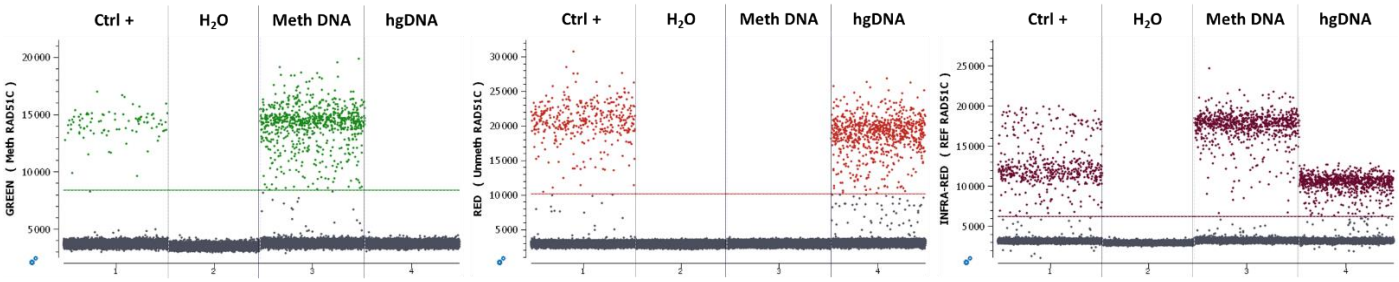

Figure 1: 1D plots obtained during wet lab testing on the 6-color naica® system. *The thresholds should be set at approximately equal distance from the positive and negative clusters.*

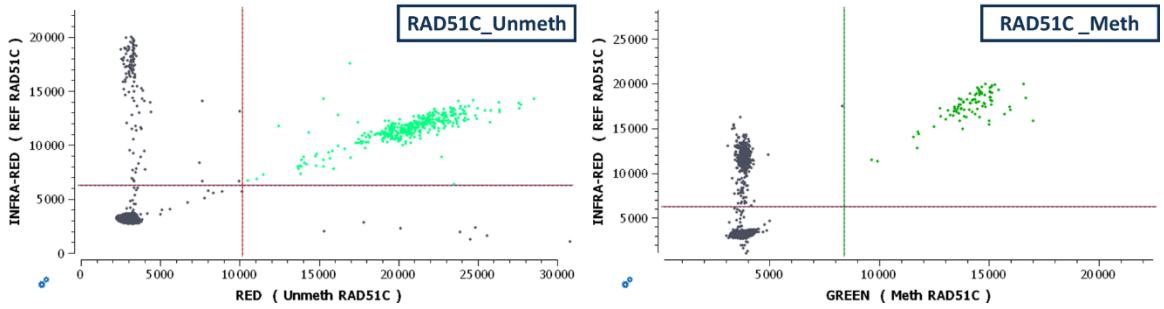

Figure 2: 2D plots obtained during wet lab testing on the 6-color naica® system. *The Red/Infra-Red double positive population corresponds to unmethylated DNA while the Green/Infra-Red double positive population corresponds to methylated DNA.*

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