Crystal Digital PCR® Assay

Information Sheet

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

Product Name

C. albicans Crystal Digital PCR® Assay (R52002)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
C. albicans	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red	16

C. albicans Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 16 genes (1 gene per chromosome) of Candida albicans using the Ruby Chip. Candida albicans is a common yeast within the human gastrointestinal microbiota. Under certain conditions, it can become pathogenic leading to infections. Treatment of C. albicans cells with antifungal drugs can drive a transient increase in ploidy and provide resistance.

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:

Chromosome	Gene	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
CHR 1 Left	ZCF23			X	X			
CHR 1 Right	CPH1					X	X	
CHR 2 Left	ERG24		X	X				
CHR 2 Right	CCT2	X		X				
CHR 3 Left	RAD53	X	X					
CHR 3 Right	AAP1				X	X		
CHR 4 Left	CPD2	X					X	
CHR 4 Right	SSK2		X		X			
CHR 5 Left	RPN8			X			X	
CHR 5 Right	RIX7	X			X			
CHR 6 Left	RPN8		X			X		
CHR 6 Right	CST5		X				X	
CHR 7 Left	C7_01960W_A	X				X		
CHR 7Right	CUP9				X		X	
CHR R Left	TRK1						X	
CHR R Right	VPS21			X		X		

Components

C. albicans 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
C. albicans Crystal Digital PCR® Assay	R52002	10X	Detects 16 genes (1 per chromosome) of Candida albicans
C. albicans Positive Control	R52002.PC0	10X	Contains: Synthetic gene target sequences (16 genes)

Specific Recommendation Regarding DNA Input

As the 16 amplicons will theoretically be amplified in each sample, it is recommended not to exceed a DNA concentration in the Ruby chamber of 250 cp/µL, which corresponds to 7.84 pg/µL or 47 pg in the 6µL 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 45 seconds	1°C/sec
Step 4	Temperature 58°C for 900 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 62°C for 45 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_Calb_R52002.ncx (6-color naica® system)
- NioProtocol_6C-60X-62°C-45s.nioprotocol (Nio™ Digital PCR)
- NioAssay_6C_Calb_R52002.nioassay (Nio™ Digital PCR)

Image Analysis

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

- CompensationMatrix_Prism6_Calb_R52002.ncm (6-color naica® system)
- CompensationMatrix_Nio_Calb_R52002.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_Calb_R52002.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

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
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***

Note that the use of a restriction enzyme (RE) may improve the separability between the positive and negative clusters. For optimal results, a final concentration of 3.6 units (U) of RE per reaction (6 µL) is suggested, and the enzyme can be directly added to the reaction mix. The restriction enzymes HindIII and EcoRI have been validated for use in this system, as they do not cleave within the DNA sequences used to design the assay.

Representative Data and Instructions for Analysis

Set thresholds for separating positive and negative populations on the 1D plots. To optimize the analysis, the thresholds should be set at approximately equal distance from the positive and negative clusters. Examples of results obtained on the Nio™+ system are given below.

Wet lab testing was carried out using a strain of *C.albicans* (SC5314), a strain of *C.tropicalis* and H_2O as negative controls and a positive control consisting of the 16 synthetic gene target sequences at 150 cp/µL in the chambers. The samples were treated with 3.6 U of HindIII per reaction as mentioned above.

Note that a few positive droplets may appear in the negative controls. However, this in no way alters the analysis, as the majority of targets should be detected in color combination (except the CHRR-L in infra-red only).

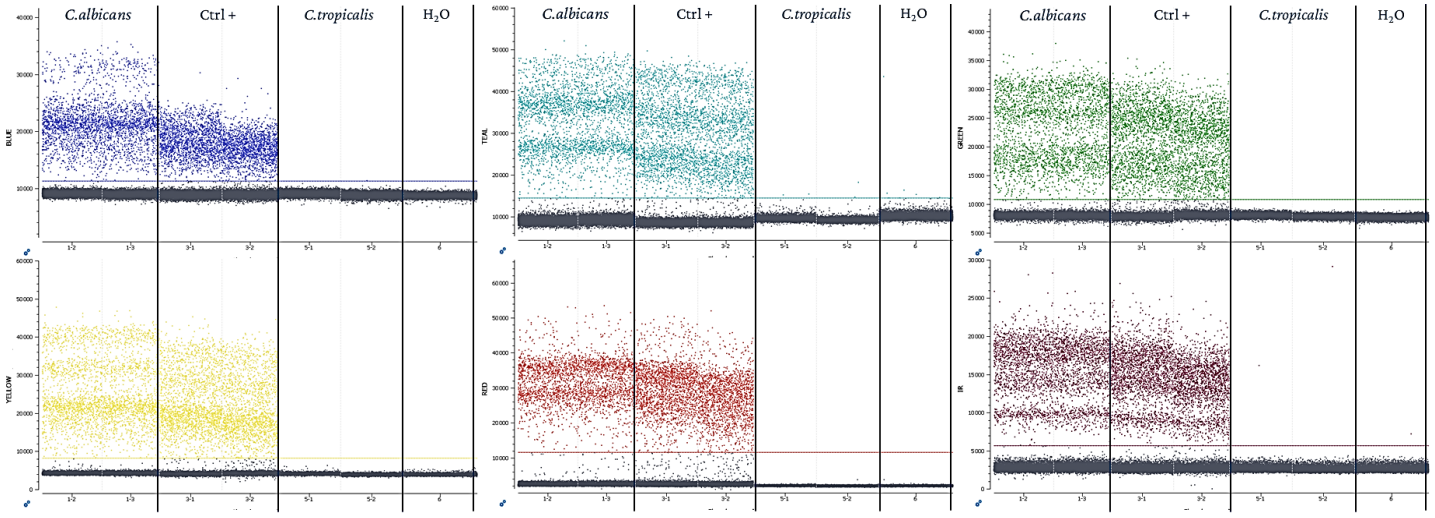

Figure 1: 1D plots obtained during wet lab testing on the Nio™+. *The thresholds are set, using the positive control, at approximately equal distance from the positive and negative clusters.*

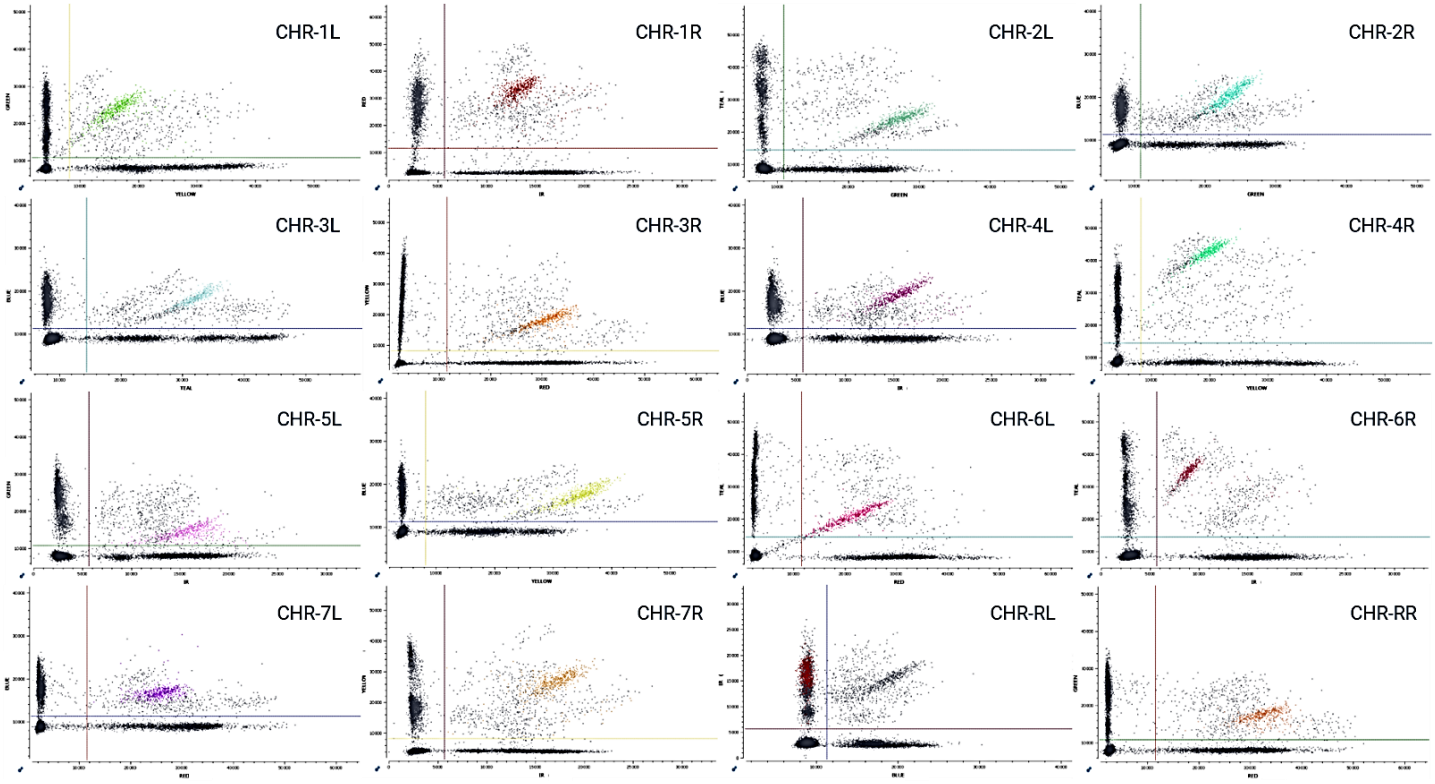

Figure 2: 2D plots obtained with the positive control during wet lab testing on the Nio™+. *Each target can be visualized as a double-positive population except CHR-RL positive only in the infra-red channel.*

Stilla Technologies
F-94800 Villejuif, FRANCE