Crystal Digital PCR® Assay

Information Sheet

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

Product Name

TERT (Reference, SLC12A7, TSN, C228T, C242T-243T, C250T) Crystal Digital PCR® Assay (R51024)

Description

Detected Targets

Targets	Sample Type	Detection Channels	Multiplex
TERT Reference, SLC12A7, TSN, C228T, C242T-243T, C250T	DNA	Blue/Teal/Green/ Yellow/Red/Infra-Red	6

TERT (Reference/SLC12A7/228T/C242T-C243T/C250T/TSN) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 3 mutations in the TERT promoter using the Ruby Chip. Three references are detected and used to quantify TERT gene amplification: a reference on the TERT gene, a reference on a gene located on the same chromosome as TERT (SLC12A7) and a reference on a different chromosome (TSN). TERT plays a critical role in cellular mechanisms linked to cancer progression, notably in maintaining telomere length and promoting immortalization.

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	Base changes	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
Reference (REF) TERT	N/A	X						
SLC12A7	N/A		X					
TSN	N/A						X	
TERT C228T	c.-124C > T	X		X				
TERT C242T-C243T	c.-138/c.-139 C > T	X			X			
TERT C250T	c.-146C > T	X				X		

Components

TERT (Reference, SLC12A7, TSN, C228T, C242T-243T, C250T) 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 upon demand to Stilla's Technical Support team at support-stilla@bio-rad.com.

Component Name	Reference	Concentration	Description
TERT (Reference/ SLC12A7/228T/C242T-C243T /C250T/TSN) Crystal Digital PCR® Assay	R51024	10X	Detects 3 mutations in the TERT promoter
TERT Positive Control	R51024.PC0	10X	Contains: hgDNA, Synthetic TERT mutants (C228T, C242T-C243T, C250T)

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 62°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 62°C for 60 seconds	2°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

Data Acquisition

Download Nio dedicated technical files from bio-rad.com.

- NioProtocol_6C-60X-62°C-60s+58°C300s.nioprotocol (Nio Digital PCR)
- NioAssay_6C_TERT_R51024.nioassay (Nio Digital PCR)

Download naica dedicated technical files from bio-rad.com.

- ScanningTemplate_Prism6_TERT_R51024.ncx (6-color naica system)

Data Analysis

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

- CompensationMatrix_Prism6_TERT_R51024.ncm (6-color naica system)
- CompensationMatrix_Nio_TERT_R51024.ncm (Nio Digital PCR)
- AnalysisConfiguration_TERT_R51024.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
- DMSO (Sigma-Aldrich Reference: D8418)
- 7-deaza-dGTP (Roche, Sigma-Aldrich Reference: 10988537001)

Instruction for PCR Mix Preparation

To ensure good assay performance, two enhancers must be added to the PCR mix: DMSO (4% vol./vol.) and 7-deaza-dGTP (200 µM). 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 Flex Probes 🟠	10x	1x	0.60
Crystal Universal Reporter Tube A ⚪	40x	1x	0.15
Crystal Universal Reporter Tube B 🟤	40x	1x	0.15
DMSO	100%	4%	0.24
7-deaza-dGTP	10mM	0.2mM	0.12
Nuclease-free water	NA	NA	Variable
Template DNA	**NA**	**NA**	**Variable**
or Positive Control ⚪	**10x**	**1x**	**0.60**
		Total reaction volume (µL)	***6.0***

DNA Digestion

DNA samples with ≥10 kb average length (e.g., genomic DNA) could be fragmented by restriction digestion before partitioning to ensure even distribution of the DNA template during partitioning. Restriction digestion is not required

for highly fragmented DNA (e.g., FFPE DNA or circulating DNA). This step could improve assay performance and should be tested utilizing desired samples.

Care must be taken to use restriction enzymes that do not cut within the amplified sequence or the Crystal Flex Probes.

For a list of restriction enzymes compatible with a given Crystal Digital PCR® assay, contact our Technical Support team (support-stilla@bio-rad.com).

Loading Amount

For optimal performance, it is recommended not to exceed a chamber concentration (DNA concentration in the reaction mix) of 1,000 copies/µL. The performance of the assay at higher concentrations is not guaranteed and must be validated by the user.

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 genomic hgDNA and H_2O as negative controls and a positive control consisting of hgDNA and 3 synthetic TERT mutants (C228T, C242T-C243T, C250T). Synthetic TERT mutants were also tested individually (C228T, C242T-C243T, C250T).

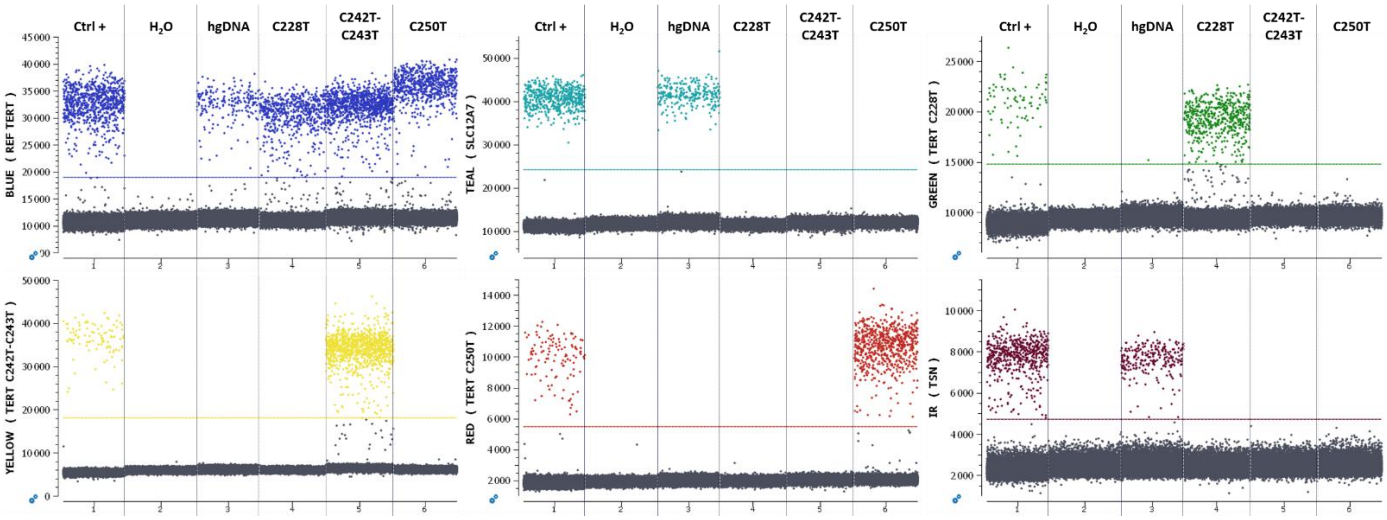
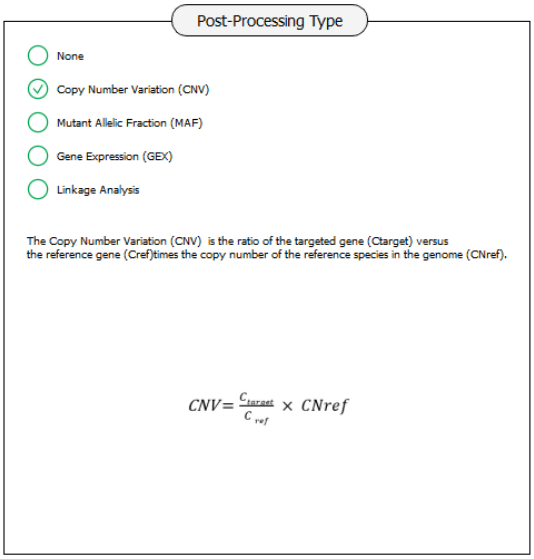
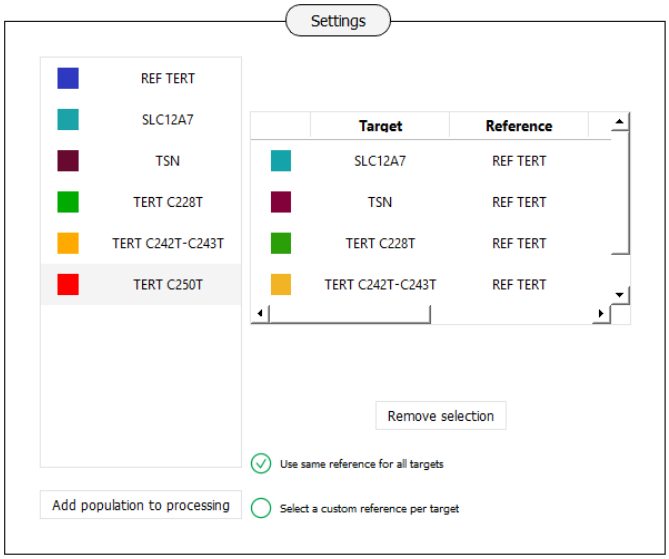


Figure 1: 1D plots obtained during wet lab testing on the Nio+. *The thresholds are set at approximately equal distance from the positive and negative clusters.*

Post-Processing (only available with NioAnalyzer software)

To perform a post-processing analysis of the results, click on “Setup” in the “POST PROCESSING” menu and select the appropriate analysis: **Copy Number Variation (CNV)**. Follow specific instructions for this assay:





All populations should be added to processing, and “REF TERT” selected as reference.

Clicking on apply will launch the calculation. The values will be displayed in the “Results” tab.

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