# Crystal Digital PCR® Assay

## Information Sheet

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

## Product Name

LBx QC Crystal Digital PCR® Assay (R51044)

## Description

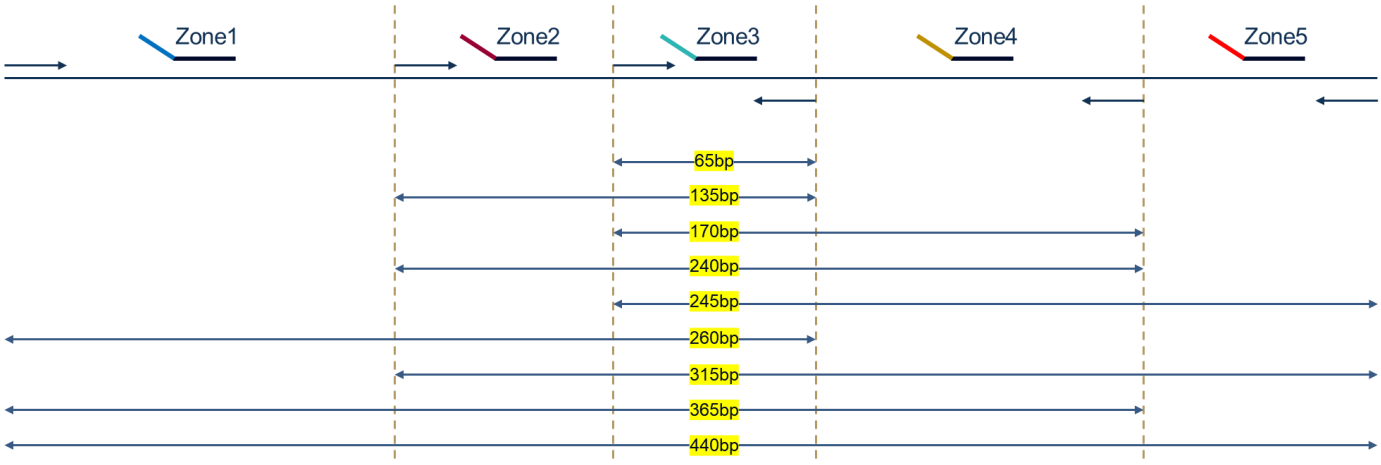
### Detected LBx QC Crystal Digital PCR® Assay Targets

| Targets | Sample Type | Detection Channels | Multiplex |
|---|---|---|---|
| **LBx QC Crystal Digital PCR® Assay – DNA lengths and Extraction Control** | DNA | Blue/Teal/Green/ Yellow/Red/Infra-Red | 6 |

Circulating cell free DNA (cfDNA) has become a monitored biomarker used for different applications such as cancer detection and monitoring. The LBx QC Crystal Digital PCR® Assay is a 10X multiplexed assay designed to quantify cfDNA and human genomic DNA (hgDNA) extracted from plasma. It gives an estimation of DNA size and an estimation of extraction yield thanks to an Extraction Control (*see Assay Protocol and Instructions for Analysis sections for more details*).

### Assay configuration

The table below indicates with a “X” which channel(s) are used for each fragment length and Extraction Control in the assay:



| Targets | Blue | Teal | Green | Yellow | Red | Infra-Red | Long-Shift |
|---|---|---|---|---|---|---|---|
| **Extraction Control** | | | X | | | | |
| **65bp fragment** | | X | | | | | |
| **135bp fragment** | | X | | | | X | |
| **170bp fragment** | | X | | X | | | |
| **240bp fragment** | | X | | X | | X | |
| **245bp fragment** | | X | | X | X | | |
| **260bp fragment** | X | X | | | | X | |
| **315bp fragment** | | X | | X | X | X | |
| **365bp fragment** | X | X | | X | | X | |
| **440bp fragment** | X | X | | X | X | X | |

### Components

LBx QC Crystal Digital PCR® Assay comprises three types of reagents:

1. A pool of extraction control,
2. A pool of the target specific primers and Crystal Flex Probes,

- 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 |
|---|---|---|---|
| **LBx QC Extraction Control** | R51044.EC | Approx. 3000 cp/µL (real concentration indicated on the tube) | Synthetic DNA to be spiked in each plasma before extraction procedure. |
| **LBx QC cdPCR Assay** | R51044 | 10X | Contains the pool of specific primers and Crystal Flex Probes for each corresponding LBx QC Assay target. |
| **LBx QC Positive Control** | R51044.PC0 | 10X | It contains human genomic DNA and synthetic DNA for extraction control target. |

## 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 40 Iterations | - |
| **Step 3.1** | Temperature 95°C for 15 seconds | 2°C/sec |
| **Step 3.2** | Temperature 64°C for 90 seconds | 2°C/sec |
| **Step 4** | Temperature 55°C for 600 seconds | 1°C/sec |
| **Step 5** | Release for Ruby Chip | - |

## Data Acquisition

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

- NioProtocol_6C-40X-64°C-90s+55°C600s.nioprotocol (Nio Digital PCR)
- Nioassay_LBx-QC-Assay_R51044.nioassay (Nio Digital PCR)

## Data Analysis

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

- CompensationMatrix_Nio_LBx-QC-Assay_R51044.ncm (Nio Digital PCR)
- AnalysisConfiguration_LBx-QC-Assay_R51044.nca (Nio Digital PCR)

## Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 7 (R42401 200 reactions, R42402 1000 reactions)
- Nuclease-free water

## Assay Protocol

### Extraction control

The addition of the Extraction Control DNA before cfDNA extraction allows to evaluate the extraction yields.

- Add 10µL (corresponding to approximately 30000* non-human DNA copies) of the Extraction Control DNA in each plasma to be extracted, whatever the plasma volume to extract.
- Start the extraction by following the usual steps of the protocol.

### 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* |

*For the clinical sample analysis, 2.4µL in Ruby reaction is the cfDNA volume recommended. But a different volume of clinical sample can be used, adapt the nuclease free water volume accordingly to assure a total volume of 6µL per Ruby reaction.

## 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 just above the negative group for all channels except for the Green channel, for which the threshold should be set at approximately equal distance from the positive and negative clusters.

Wet lab testing was carried out using human genomic DNA (hgDNA), $H_2O$ as negative control, a positive control consisting of hgDNA and the 1 synthetic target sequence (Extraction Control), two cfDNA after extraction with Maxwell® Instrument from Promega, and the Extraction Control.

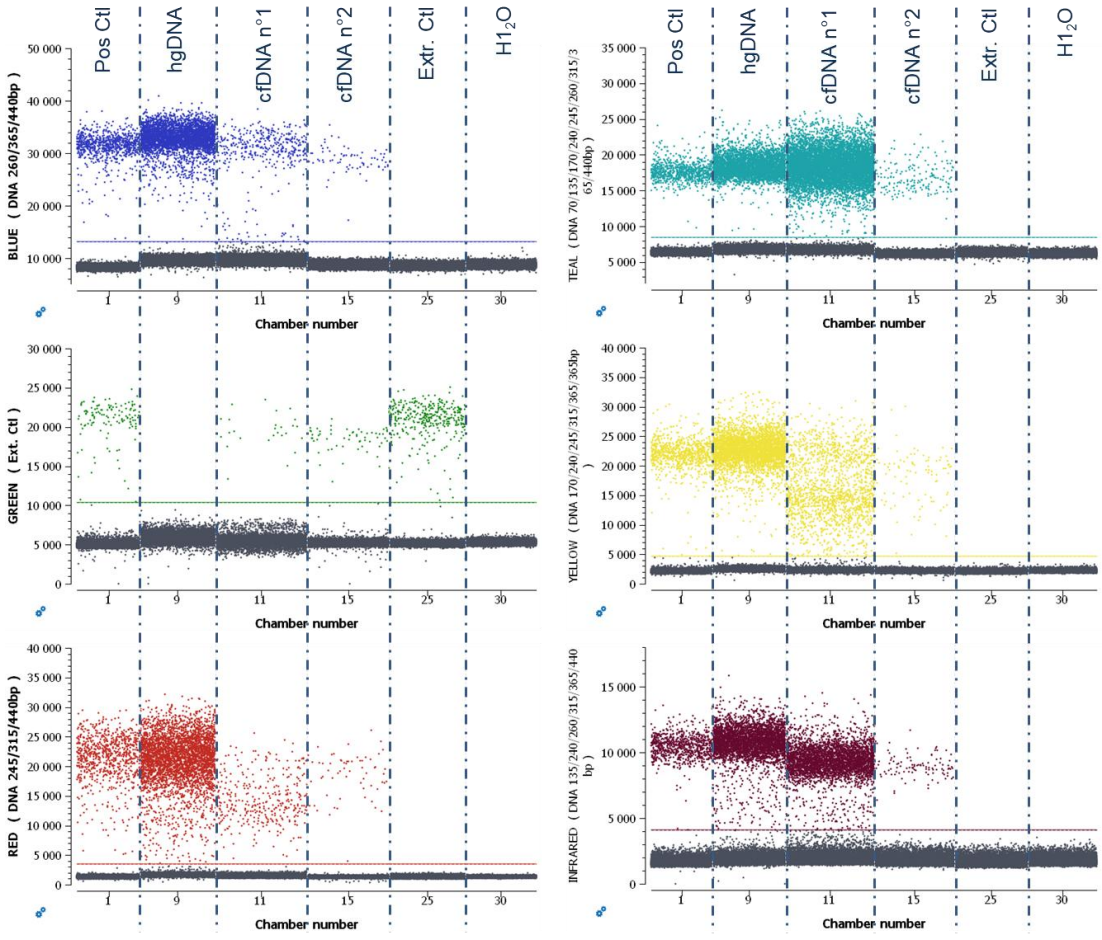



***Figure 1: 1D plots obtained during wet lab testing on the Nio+.*** *The thresholds are set, based on positive control, just above the negative group for all channels except for the Green channel, for which the threshold should be set at approximately equal distance from the positive and negative clusters.*

## Calculation of concentration and proportion of each DNA fragment

To determine the concentration and percent of each DNA fragment, download the dedicated **Excel Analysis Workbook** from the Technical Resources section of the Stilla Technologies website and follow the instructions given on the first worksheet:

- PostProcessing_LBx-QC-Assay_R51044.xlsx

## Calculation of extraction yield

The **extraction yield** of each cfDNA can be evaluated as follows:

$$\textbf{Extraction yield} = \frac{[Extraction\ control\ DNA]}{\left(\frac{Copy\ number\ of\ Extraction\ control\ DNA}{Velu}\right)} * \frac{Total\ volume\ reaction\ mix}{Input\ DNA\ volume}$$

- [Extraction control DNA] = concentration of the Extraction Control in copies/µL after the dPCR run.
- Copy number of Extraction control DNA = initial concentration of the extraction Control (approx. 3000 cp/µL) * Volume added in plasma before extraction = 3000cp/µL * 10µL = 30000 copies
- $V_{elu}$ = Volume of elution buffer used to eluate cfDNA at the end of extraction procedure.
- Total volume reaction mix = 6µL for 1 Ruby reaction.
- Input DNA volume = volume of DNA added in reaction mix.

Once yield has been evaluated, the corrected concentrations of each fragment can be calculated by dividing the concentrations by the extraction yield.



Stilla Technologies
F-94800 Villejuif, FRANCE