

# Crystal Digital PCR® Assay

## Information Sheet

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

### Product Name

Meat (Beef, Pork, Chicken, Sheep) 4plex Crystal Digital PCR® Assay (R54002)

### Description

#### Detected Targets

Targets	Detection Channels	Multiplex
<b>Beef (Bos taurus)</b> <b>Pork (Sus scrofa)</b> <b>Chicken (Gallus gallus)</b> <b>Sheep (Meleagris gallopavo)</b>	Blue / Green / Yellow / Red	4

The Meat 4plex Crystal Digital PCR® Assay is a highly sensitive 10X assay designed to detect and quantify 4 meat species from Beef, Pork, Chicken, and Sheep.

#### Assay Configuration

The table below indicates with a “X” which channel(x) are used for each target in the assay:

Targets	Blue	Teal	Green	Yellow	Red	Infra-Red	Long-Shift
<b>Beef (Bos taurus)</b>	X						
<b>Pork (Sus scrofa)</b>			X				
<b>Chicken (Gallus gallus)</b>					X		
<b>Sheep (Meleagris gallopavo)</b>				X			

#### Components

The assay comprises two reagents: a pool of the assay specific primers and Crystal Flex Probes and a pool of 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](mailto:support-stilla@bio-rad.com).

Component Name	Reference	Concentration	Description
<b>Meat (Beef, Pork, Chicken, Sheep) 4plex Crystal Digital PCR® Assay</b>	R54002	10X	Detection of the 4 meats (Beef, Pork, Chicken, Sheep)
<b>Meat 4plex Positive Control</b>	R54002.PC0	10X	Contains synthetic DNA of the 4 targets

## Before starting the experiment

Prior to creating your first experiment using PerfeCTa® Multiplex qPCR ToughMix® on a compatible system, please launch the installer for the PerfeCTa® Multiplex qPCR ToughMix®. It is available upon request to the Stilla's Technical Support team.

## 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 60 Iterations	-
Step 3.1	Temperature 95°C for 15 seconds	2°C/sec
Step 3.2	Temperature 60°C for 30 seconds	2°C/sec
Step 4	Temperature 58°C for 300 seconds	1°C/sec
Step 5	Release for Ruby Chip	-

### 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 58°C for 30 seconds	1°C/sec
Step 4	Release for Ruby Chip	-

## Data Acquisition

Download Nio dedicated technical files from [bio-rad.com](http://bio-rad.com).

- NioProtocol\_6C-60X-60°C-30s\_Meat.nioprotocol (Nio Digital PCR)
- NioAssay\_6C\_Meat.nioassay (Nio Digital PCR)

Download naica dedicated technical files from [bio-rad.com](http://bio-rad.com).

- ScanningTemplate\_Prism6\_6C\_Meat\_R54002.ncx (6-color naica system)

## Data Analysis

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

- CompMatrix\_Meat\_6C\_Nio.ncm (Nio Digital PCR)
- AnalysisConfiguration\_Meat.nca (Nio Digital PCR)
- MeanCompMatrix\_Meat\_6C\_Naica.ncm (6-color naica system)
- AnalysisConfiguration\_Meat\_naica.nca (6-color naica system)

## Consumables Required but Not Provided

- Ruby Chip (C16011)
- PerfeCTa® Multiplex qPCR ToughMix® - Quantabio, references: 95147-250, 95147-01K, 95147-05K
- Crystal Universal Reporters 7 (R42401 200 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)
PerfeCTa® Multiplex qPCR ToughMix®	5x	1x	1.20
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>

## DNA Digestion

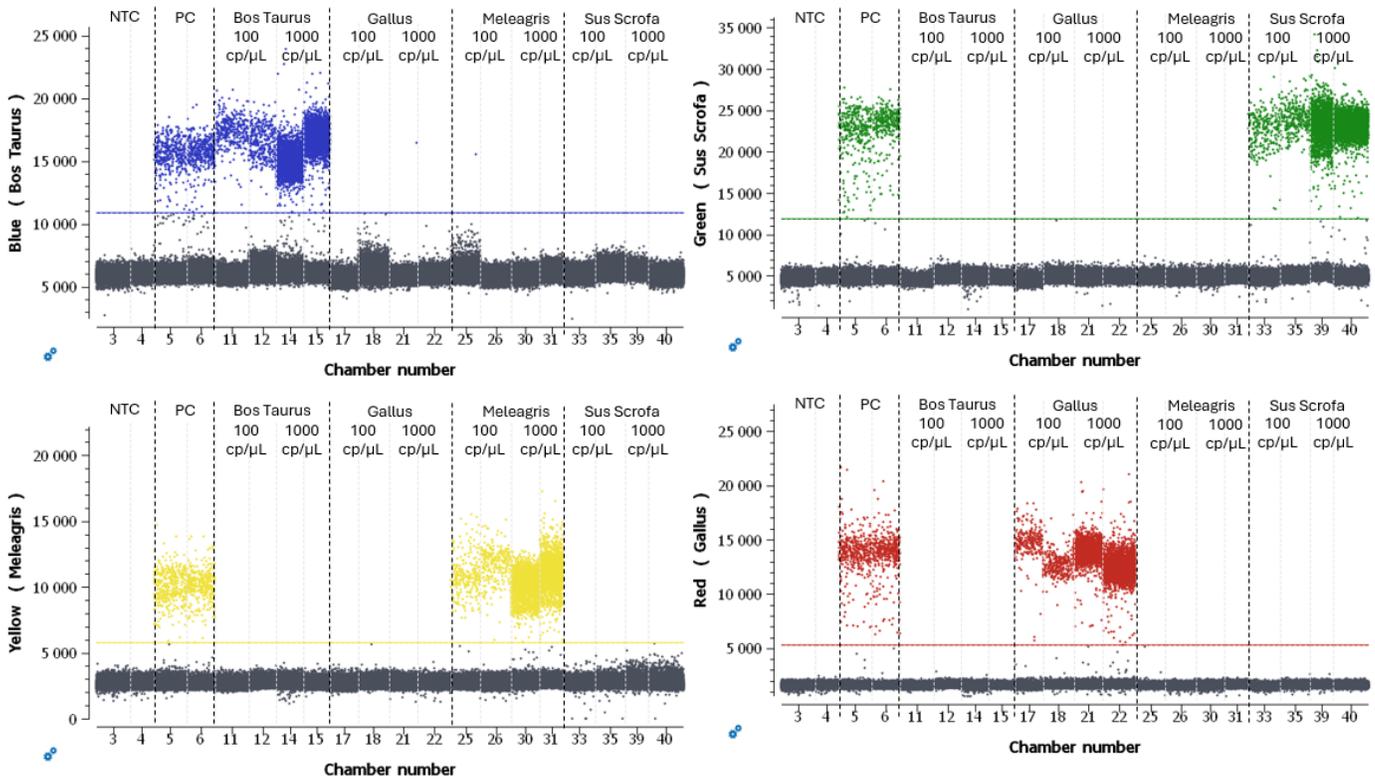
DNA samples with ≥10 kb average length (e.g., genomic DNA) could be fragmented by restriction digestion before partitioning to ensure even distribution of the DNA template during partitioning. Restriction digestion is not required for highly fragmented DNA (e.g., FFPE DNA or circulating DNA). This step 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](mailto:support-stilla@bio-rad.com)).

## 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 are given below.



**Figure 1: 1D plots obtained during testing on the Nio+.** The thresholds should be set at approximately equal distance from the positive and negative clusters. A non-template control (NTC), a positive control (PC) containing synthetic DNA for the 4 targets, and each target individually at 100 and 1000 copies/μL (cp/μL) have been tested.

AIS\_R54002\_v1



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