# Crystal Digital PCR® Assay

## Information Sheet

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

## Product Name

MYD88 (L265, L265P) Crystal Digital PCR® Assay (R51015)

## Description

### Detected Targets

| Targets | Sample Type | Detection Channels | Multiplex |
|---|---|---|---|
| **MYD88 (L265, L265P)** | DNA | Blue/Red | 2 |

The MYD88 (L265, L265P) Crystal Digital PCR® Assay is a 10X assay designed to detect and quantify 1 mutation in the MYD88 gene using the Ruby Chip. MYD88 encodes the MYD88 protein, which is an adaptor protein essential for transmitting signals in the immune system, mediating responses to infections, and modulating inflammation and cell survival.

### Assay Configuration

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 |
|---|---|---|---|---|---|---|---|---|
| **Wild-type (WT) MYD88 L265** | N/A | X | | | | | | |
| **MYD88 L265P** | c.794T>C | | | | | X | | |

### Components

MYD88 (L265, L265P) 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 |
|---|---|---|---|
| **MYD88 (L265, L265P) Crystal Digital PCR® Assay** | R51015 | 10X | Detects 1 mutation in the MYD88 gene |
| **MYD88 Positive Control** | R51015.PC0 | 10X | Contains: hgDNA and synthetic MYD88 L265P mutant |

## 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 58°C for 30 seconds | 1°C/sec |
| **Step 4** | 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 30 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_Prism3_MYD88_R51015.ncx (3-color naica® system)
- ScanningTemplate_Prism6_MYD88_R51015.ncx (6-color naica® system)
- NioProtocol_3C-60X-60°C-30s.nioprotocol (Nio™ Digital PCR)
- NioAssay_3C_MYD88_R51015.nioassay (Nio™ Digital PCR)

## Image Analysis

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

- CompensationMatrix_Prism3_MYD88_R51015.ncm (3-color naica® system)
- CompensationMatrix_Prism6_MYD88_R51015.ncm (6-color naica® system)
- CompensationMatrix_Nio_MYD88_R51015.ncm (Nio™ Digital PCR)
- AnalysisConfiguration_MYD88_R51015.nca (all systems)

## Consumables Required but Not Provided

- Ruby Chip (C16011)
- naica® PCR MIX 10X (R10106)
- Crystal Universal Reporters 3 (R41401 200 reactions, R41402 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 |
| 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 blue and the 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.

Wet lab testing was carried out using genomic hgDNA and $H_2O$ as negative controls and a positive control consisting of hgDNA and synthetic MYD88 L265P mutant. Synthetic MYD88 L265P mutant was also tested individually.

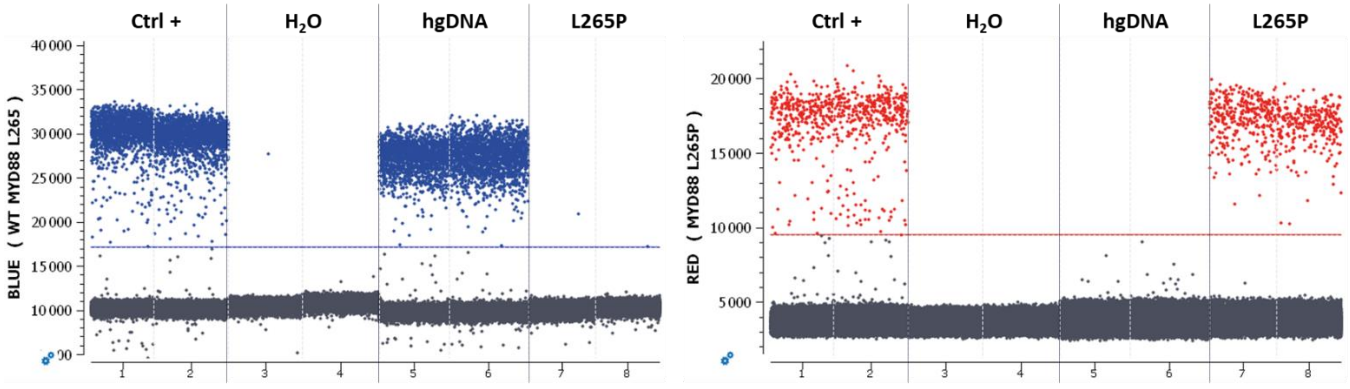


***Figure 1: 1D plots obtained during wet lab testing on the 6-color naica® system.*** *The blue and the red thresholds should be set at approximately equal distance from the positive and negative clusters.*

Stilla Technologies
F-94800 Villejuif, FRANCE