ACCURATE CRYSTAL DIGITAL PCR™ QUANTIFICATION OF A HUMAN TARGET GENE USING THE NAICA® PCR MIX AND EVAGREEN®

EvaGreen® is a sequence-independent double-stranded DNA intercalating dye with low fluorescence when free in solution and high fluorescence when bound to DNA. Using EvaGreen® on the naica® system enables absolute quantification of a DNA target using a simple primer pair and represents a low-cost solution when simultaneous quantification of multiple individual targets is not required. The naica® PCR MIX is a PCR master mix specially formulated to ensure robust partition compatibility and excellent linear Crystal Digital PCR™ quantification optimized for EvaGreen® on the naica® system.

LINEAR QUANTIFICATION ACROSS A 4-LOG DILUTION RANGE USING THE NAICA® PCR MIX

The naica® PCR MIX is specially formulated for Crystal Digital PCR™ detection and quantification with the naica® system using EvaGreen® dye chemistry. In the presence of varying levels of fluorescence background signal, the naica® PCR MIX allows reliable quantification of human genomic DNA (hgDNA) across a 4-log dilution range (**Figure 1**).

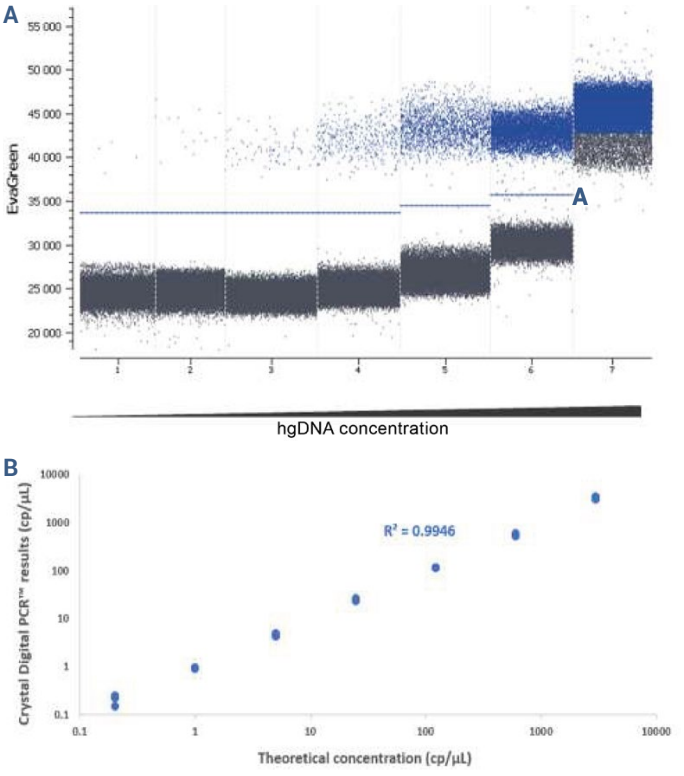

Figure 1: Linearity and sensitivity of absolute quantification of hgDNA by Crystal Digital PCR™ using the 10X naica® PCR MIX and a final reaction concentration of 1.9 µM EvaGreen®. (A) 1D-dotplot generated by Crystal Miner software showing robust positive and negative cluster separability from 16.5 pg (0.2 copies (cp)/µL) up to 248 ng (3000 cp/µL) of hgDNA in a 25 µL reaction using Sapphire chips. (B) Each dilution point from 0.2 cp/µL to 3000 cp/µL of hgDNA was assessed in triplicates. A coefficient of determination score of R^2>0.99 demonstrates excellent reliability of the EvaGreen® analysis with the naica® PCR MIX. The hgDNA concentrations of each dilution point were: 0.2, 1.0, 5.0, 25, 120, 600, and 3000 cp/µL.

HIGHER EVAGREEN® FINAL CONCENTRATIONS ALLOW IMPROVED QUANTIFICATION OF CONCENTRATED TARGETS

In the presence of high concentrations of DNA, the signal from the proportion of EvaGreen® dye bound to the amplicon could become difficult to distinguish from the background signal contributed by the proportion of EvaGreen® dye bound to the template DNA. By increasing the final reaction concentration of EvaGreen® from 1.9 µM to 3.7 µM, the signal to noise ratio was improved (**Figure 2**).

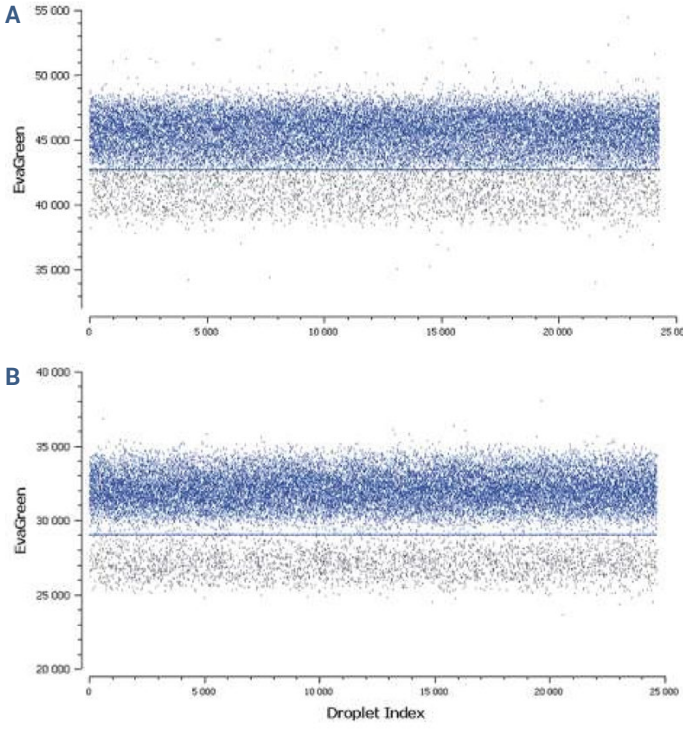

Figure 2: 1D-fluorescence dotplots of Crystal Digital PCR™ performed with 10X naica® PCR MIX and final reaction concentrations of **(A)** 1.9 µM EvaGreen® versus **(B)** 3.7 µM EvaGreen® for quantification of 3000 cp/µL (248 ng) of hgDNA. Increasing the EvaGreen® final concentration by two-fold allows a cleaner separation of positive and negative fluorescence clusters and ensures robust thresholding.

Compared to conventional EvaGreen®-compatible PCR master mixes, the naica® PCR MIX is available at high initial concentrations (5X and 10X), freeing up valuable reaction volume that can be instead occupied by additional sample input and/or increased EvaGreen® final concentrations up to 3.7 µM.

TECHNICAL NOTE HIGHLIGHTS

- The naica® PCR MIX enables an excellent linear Crystal Digital PCR™ detection across 4-log dilutions using EvaGreen® dye chemistry.
- naica® PCR MIX is available in two concentration formats, 5x and 10x concentrations, maximizing the reaction volume that can be occupied by a sample.
- naica® PCR MIX allows EvaGreen® concentration optimization increasing the detection sensitivity and resulting in improved assay separability.

To learn more about digital PCR, please visit Stilla Technologies' Learning Center at **stillatechnologies.com/digital-pcr**