Fluidigm[®] 12.765 Digital PCR Workflow Quick Reference

PN 68000096, Rev. D

For more information see the BioMark Digital PCR Analysis Software User Guide, PN 68000100

Priming the 12.765 Digital Array™ IFC

CAUTION! USE THE 12.765 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE $48.48~\mbox{syringes}$ with $300~\mbox{\mu}L$ of control line fluid.
- CONTROL LINE FLUID ON THE CHIP OR IN THE INLETS MAKES THE CHIP
 UNUSABLE.
- LOAD THE CHIP WITHIN 60 MINUTES OF PRIMING.
- 1 Inject control line fluid into each accumulator on the chip.
- 2 Remove and discard the blue protective film from the bottom of the chip.
- 3 Place the chip into the IFC (Integrated Fluidic Circuit) Controller MX, then run the Prime (115x) script to prime the control line fluid into the chip.

Preparing Sample Pre-Mix and Samples

1 Combine the components in the table below to make the Sample Pre-Mix and the final Sample Mixture (scale up appropriately for multiple runs).

	Component	Volume per Inlet (μL)	Volume per Inlet with Overage (μL)	Volume per Chip (µL) (enough for 13 reactions)
PRE-MIX	TaqMan [®] Gene Expression Master Mix (Applied Biosystems, PN 4369016)*	4.0	5	65
- H	20X GE Sample Loading Reagent (Fluidigm, PN 85000746)	0.4	0.5	6.5
SAM	20X gene-specific assays	0.4	0.5	6.5**
	DNA-free water	2.4	3	39
	DNA	0.8	1	
	Total	8	10	117

* TaqMan[®] Universal PCR Master Mix (Applied Biosystems, PN 4304437) may be substituted. Fluidigm recommends using TaqMan Gene Expression Master Mix for the Digital Array IFC.

**The 20X assay can be removed from the Sample Pre-Mix and added separately if different assays are to be used on the same chip.

- 2 In a DNA-free hood, combine the Taqman Gene Expression Master Mix, GE Sample Loading Reagent, DNA-free water and 20X assay in a 1.5 mL sterile tube—enough volume to fill the entire chip. 9.0 μL of this Sample Pre-Mix can then be aliquoted for each sample (12 total).
- 3 Remove these aliquots from the DNA-free hood and add 1.0 µL of DNA to each, making a total volume of 10 µL in each aliquot.

Loading the Chip

IMPORTANT! VORTEX THOROUGHLY AND CENTRIFUGE ALL SAMPLE SOLUTIONS BEFORE PIPETTING INTO THE CHIP INLETS. FAILURE TO DO SO MAY RESULT IN A DECREASE IN DATA QUALITY.

CAUTION! WHILE PIPETTING, DO NOT GO PAST THE FIRST STOP ON THE PIPETTE. DOING SO MAY INTRODUCE AIR BUBBLES INTO THE INLETS.

- 1 When the **Prime (115x)** script has finished, remove the primed chip from the IFC Controller MX.
- 2 Pipette 8 µL of DNA-free water into each H inlet.
- 3 Pipette 8 µL sample mix into the sample inlets on the chip.
- 4 Return the chip to the IFC Controller MX.
- 5 Using the IFC Controller MX software, run the Load (115x) script to load the samples into the chip.
- 6 When the Load (115x) script is complete, remove the chip from the IFC Controller MX.
- 7 Remove any dust particles or debris from the chip surface.

You are now ready for your chip run.

CAUTION! START CHIP RUN WITHIN 4 HOURS OF LOADING THE SAMPLES.

12.765 Pipetting Map

- Add 8 μL of DNA-free water
- Do NOT use
- Pipette samples into Sample inlets 1–12





Using the Data Collection Software

UNSPECIFIED PROBES 1 Double-click the Data Collection Software icon on the desktop to 8 Application, Reference, Probes: launch the software. a Select Application Type-Digital PCR. 2 Click Start a New Run. b Select Passive Reference (ROX). 3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding. More than two probes. Camera Temperature: -5.0 °C 🥥 Lamp is on d Select probe types. e Click Next. 4 Place the loaded chip into the reader.

BE SURE TO SELECT ALL PROBE TYPES PRESENT IN YOUR EXPERIMENT. DATA ARE NOT COLLECTED ON

5 Click Load.

IMPORTANT!

- 6 Verify chip barcode and chip type.
 - a Choose project settings (if applicable).
 - b Click Next.
- 7 Chip Run file:
 - a Choose New or Predefined.
 - b Choose a file location for data storage.
 - c Click Next.

- c Select Assav-Single probe, Two probes, or
- 9 Click Browse to find the appropriate thermal protocol file-dPCR Standard v1.pcl.
- 10 Confirm Auto Exposure is selected.
- 11 Click Next
- 12 Verify the chip run information.
- 13 Click Start Run.
- NOTE TO RUN THIS PROTOCOL AS AN END-POINT AND USE THE FLUIDIGM STAND-ALONE THERMAL CYCLER OR THE FLUIDIGM FC1[™] CYCLER, REFER TO THE *FLUIDIGM STAND-ALONE THERMAL CYCLER USAGE* QUICK REFERENCE (PN 68000111) OR THE *FLUIDIGM FC1 CYCLER* USAGE QUICK REFERENCE (PN 100-1250), RESPECTIVELY.

Using the Digital PCR Analysis Software

IMPORTANT! BE SURE TO CLICK ANALYZE EACH TIME YOU CHANGE A PARAMETER IN THE SOFTWARE.

- Double-click the Digital PCR Analysis software icon on the 1 desktop to launch the software.
- 2 Click Open a Chip Run.
- 3 Double-click a chiprun.bml file to open it in the software.
- 4 Click Sample and Detector Setup in the Chip Explorer pane.
- 5 Click New or Import.
- 6 Highlight the wells and then annotate them.
- 7 Click Editor in the Sample and Detector Setup pane.
- Choose Sample Type from the drop-down menu in the Editor. 8
- Type a name for the sample. 9

Technical Support

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- Choose Detector Type from the drop-down menu in the Editor. 10
- 11 Type a name for the detector.
- Click Update to see the changes reflected in the highlighted wells. 12
- 13 Click Panel Summary in the Chip Explorer pane.
- Click Analyze in the Task pane. 14
- Click Panel Summary or Panel Details. 15
- Choose a view from the drop-down menu: 16
 - · Results Table
 - Image View
 - · Heat Map View

Chip Explorer	Panel Summary		
🖃 闄 Chip Run Summary		📳 Results Table 👻	
Panel Summary			Results Table
Panel01		a),	Image View
Panel02		88	Heat Map View

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