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*

1 Priming the 12.765 Digital Array™ IFC

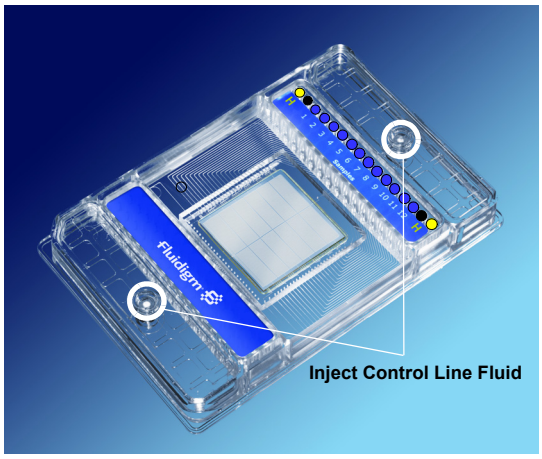
CAUTION! USE THE 12.765 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE 48.48 SYRINGES WITH 300 µL 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.



2 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)
SAMPLE PRE-MIX	TaqMan® Gene Expression Master Mix (Applied Biosystems, PN 4369016)*	4.0	5	65
SAMPLE PRE-MIX	20X GE Sample Loading Reagent (Fluidigm, PN 85000746) ●	0.4	0.5	6.5
SAMPLE PRE-MIX	20X gene-specific assays	0.4	0.5	6.5**
SAMPLE PRE-MIX	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.

3 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.

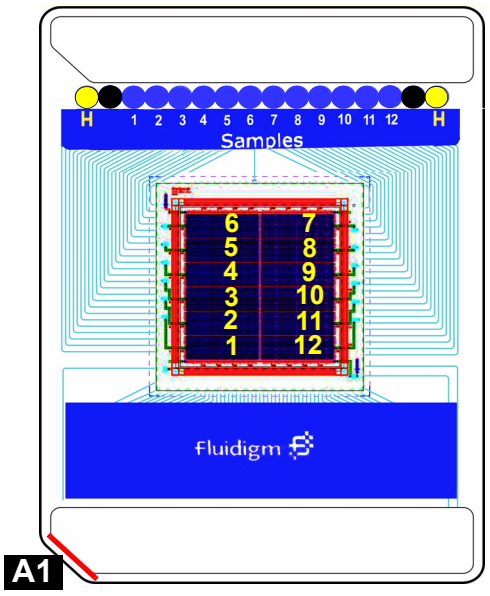
7 Remove any dust particles or debris from the chip surface.

You are now ready for your chip run.

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

12.765 Pipetting Map

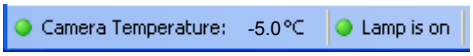
- Add 8 µL of DNA-free water
- Do NOT use
- Pipette samples into Sample inlets 1–12



4 Using the Data Collection Software

IMPORTANT! BE SURE TO SELECT *ALL* PROBE TYPES PRESENT IN YOUR EXPERIMENT. DATA ARE NOT COLLECTED ON UNSPECIFIED PROBES.

1 Double-click the Data Collection Software icon on the desktop to launch the software.
2 Click **Start a New Run**.
3 Check the status bar to verify that the lamp and the camera are ready. Make sure both are green before proceeding.



4 Place the loaded chip into the reader.
5 Click **Load**.
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**.

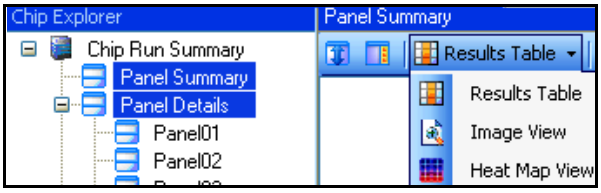
8 Application, Reference, Probes:
  a Select Application Type—**Digital PCR**.
  b Select Passive Reference **(ROX)**.
  c Select Assay—**Single probe**, **Two probes**, or **More than two probes**.
  d Select probe types.
  e Click **Next**.
9 Click **Browse** to find the appropriate thermal protocol 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.

5 Using the Digital PCR Analysis Software

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

1 Double-click the Digital PCR Analysis software icon on the 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.
8 Choose **Sample Type** from the drop-down menu in the Editor.
9 Type a name for the sample.
10 Choose **Detector Type** from the drop-down menu in the Editor.
11 Type a name for the detector.
12 Click **Update** to see the changes reflected in the highlighted wells.
13 Click **Panel Summary** in the Chip Explorer pane.
14 Click **Analyze** in the Task pane.
15 Click **Panel Summary** or **Panel Details**.
16 Choose a view from the drop-down menu:
  - Results Table
  - Image View
  - Heat Map View



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