

Digital PCR with the 12.765 IFC Using Gene-Specific Assays

IMPORTANT Before using the 12.765 Digital Array™ IFC (integrated fluidic circuit), read and understand the detailed instructions and safety guidelines in the Digital PCR Analysis User Guide (68000100), Juno System User Guide (100-7070) or IFC Controller MX and IFC Controller HX User Guide (68000112), and Biomark HD Data Collection User Guide (100-2451) or Biomark/EP1 Data Collection User Guide (68000127).

Choose a Juno/IFC Controller MX Workflow

For Real-Time dPCR

Prime	Load	Thermal-cycle (PCR) and image
Juno™ or MX	Juno or MX	Biomark™ HD or Biomark

For Endpoint Read

Prime	Load and thermal-cycle (PCR)	Image
Juno or MX	Juno one-step loading and PCR	Biomark HD or Biomark or EP1™

Prime	Load	Thermal-cycle (PCR)	Image
Juno or MX	Juno or MX	Juno or FC1™ cyclor	Biomark HD or Biomark or EP1

Prepare the Sample Pre-Mix and Samples

- 1 Briefly vortex and centrifuge reagents before use.
- 2 In a DNA-free hood, combine the components (Table 1) in a sterile tube to make the sample pre-mix. This is enough volume to fill the entire IFC. Scale up appropriately for multiple runs.

Table 1. Sample pre-mix

Component	Vol/Inlet with Overage (µL)	Vol/IFC (µL)*
TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific™, 4369016)†	5.0	65.0
20X GE Sample Loading Reagent (85000746) ●	0.5	6.5
20X gene-specific assays	0.5	6.5‡
DNA-free water	3.0	39.0
Total	9.0	117.0

* Enough for 60 reactions for ease of pipetting.

† TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, 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 you are using different assays on the same IFC.

- 3 Pipet 9.0 µL of the sample pre-mix for each sample (12 total) into a new 96-well plate.
- 4 Remove the plate from the DNA-free hood and add DNA to each well containing sample pre-mix to prepare the sample mix (Table 2).

Table 2. Sample mix

Component	Vol/Inlet with Overage (µL)
Sample pre-mix (see Table 1)	4.2
DNA	1.8
Total	6.0

- 5 Seal the plate, then vortex and centrifuge it for 60 sec.

Prime the 12.765 Digital Array IFC

IMPORTANT

- Use the IFC within 24 hr of opening the package.
- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of control line fluid (89000020).
- Control line fluid on IFC or in the inlets makes IFC unusable.
- 1 Inject control line fluid into each accumulator on the IFC (Figure 1). Use the entire contents of the syringe.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the controller:
 - Juno: Tap **OPEN** to open the instrument tray and align the notched corner of IFC to the white notch on tray. Tap **LOAD**.
 - MX: Press **EJECT** to open the instrument tray and align the notched corner of the IFC to the A1 mark. Press **Load Chip**.
- 4 Run the prime script:
 - Juno: Tap **Prime 12.765**, and then tap **Run**.
 - MX: Select **Prime (115x)** and press **Run Script**.

IMPORTANT The prime script takes approximately 5 min to run. Load the IFC within 60 min of completing the prime script.

Load the IFC

IMPORTANT

- Vortex thoroughly and centrifuge all sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.
- 1 After the prime script is finished, remove the primed IFC from the controller.
- 2 Pipet 10 µL of 1X GE Sample Loading Reagent into each hydration inlet (Figure 1).
- 3 Pipet 4 µL sample mix into each sample inlet (Figure 1).

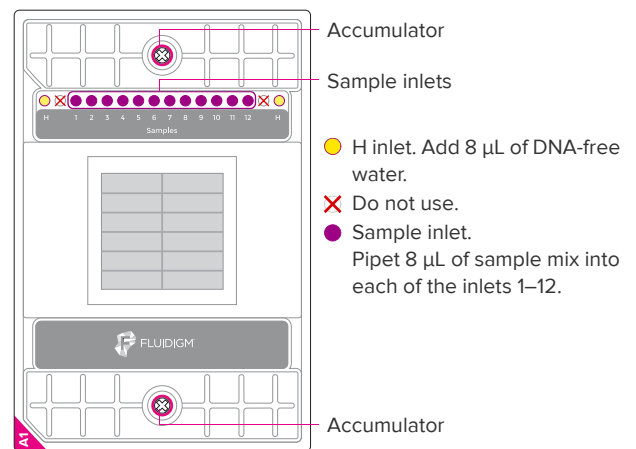


Figure 1. 12.765 IFC pipetting map

- 4 Return IFC to Juno or MX, then run script according to operation:

Instrument	Operation	Run Script	Continue to
Juno	Loading only	Load 12.765	For real-time dPCR: Collect Data
MX	Loading only	Load (115x)	For endpoint read: Thermal-Cycle the IFC (endpoint read only)
Juno (endpoint read only)	One-step loading and thermal-cycling	One Step 12.765	Collect Data

IMPORTANT

- The load script takes approximately 40 min to run. For real-time dPCR, start the IFC run on Biomark HD or Biomark within 1 hr of completing the load script. For endpoint read using the load script, thermal-cycle the IFC within 1 hr of completing the load script.
- For endpoint read using the One Step script: Start the IFC run on EP1, Biomark HD, or Biomark within 1 hr of completing the One Step 12.765 script.

Thermal-Cycle the IFC (endpoint read only)

Choose the instrument and run the script:

Instrument	Operation	Run Script
Juno	One-step loading and PCR	—
Juno	PCR only	PCR 12.765
FC1 cyclers	PCR only	dPCR Standard v1.pcl

IMPORTANT Thermal-cycling takes approximately 90 min to run. Start the IFC run on Biomark HD, Biomark, or EP1 within 1 hr of thermal-cycling samples.

Collect Data

- 1 Remove the loaded IFC from the controller or thermal-cycler.
- 2 Use clear tape to remove any dust particles or debris from the IFC surface.
- 3 If necessary, double-click the **Data Collection** icon on the desktop of the Biomark HD, Biomark, or EP1 system computer to launch the software.
- 4 Click **Start a New Run**.
- 5 Confirm that the camera status indicator and the lamp status indicator (Biomark and EP1 only) at the bottom of the window are green.
- 6 Place the loaded IFC on the instrument tray and align the notched A1 corner on the IFC with the A1 label on the tray. Click **Load**.
- 7 Confirm the IFC barcode and IFC type and then click **Next**.
- 8 Complete the Chip Run section by selecting either a new or a pre-defined run.
- 9 Complete the Chip Run Name and Location section:
 - a Enter a run name or select the checkbox to use the IFC barcode as the run name.

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- b Select a file storage location for a new IFC run or browse to select a pre-defined run file and click **Next**.

- 10 Complete the Application, Reference and Probes section and then click **Next**.

For	Select
Application	Digital PCR
Passive reference	ROX™
Assay	Single probe, Two probes, or More than two probes.
	Select probe types.
Probes	IMPORTANT Be sure to select all probe types present in your experiment. Data is not collected on unspecified probes.

- 11 Browse to and select the thermal protocol:

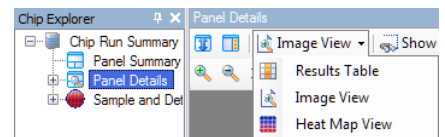
For	Select
Real-time dPCR run	dPCR Standard v1.pcl
Endpoint run after thermal cycling on Juno or FC1	dPCR End Point v1.pcl

- 12 Confirm that **Auto Exposure** is selected. Click **Next**.
- 13 Confirm that the IFC run information is correct, then click **Start Run**.
- 14 After the run is complete, process your data using the Digital PCR Analysis software.

Use the Digital PCR Analysis Software

IMPORTANT Each time you change a parameter in the software click **Analyze** to update the results.

- 1 Double-click the **Digital PCR Analysis** 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.
- 9 Enter a name for the sample.
- 10 Choose **Detector Type** from the drop-down menu.
- 11 Enter 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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