Digital PCR with the 48.770 IFC Using Gene-Specific Assays

IMPORTANT Before using the 48.770 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™ cycler	Biomark HD or Biomark or EP1

Prepare 1X GE Sample Loading Reagent

☐ **1** Combine components (Table 1) in a sterile tube and set aside until you are ready to load the IFC.

Table 1. 1X GE Sample Loading Reagent

Component	Vol/Inlet with overage (µL)
20X GE Sample Loading Reagent (85000746) ●	10
DNA-free water	190
Total	**200**

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 2) 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.

NOTE For a copy number variation application, substitute 20X RNase P Assay for the DNA-free water.

Table 2. Sample pre-mix

Component	Vol/Inlet with Overage (µL)	Vol/IFC (µL)*
TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific™, 4369016)†	3.0	180
20X GE Sample Loading Reagent (85000746) ●	0.6	36
20X gene-specific assays	0.3	18‡
DNA-free water	0.3	18
Total	**4.2**	**252**

* 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 4.2 µL of the sample pre-mix for each sample (48 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 3).

Table 3. Sample mix

Component	Vol/Inlet with Overage (µL)
Sample pre-mix (see Table 2)	4.2
DNA	1.8
Total	**6.0**

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

Prime the 48.770 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 48.770**, and then tap **Run**.
- MX: Select **Prime (148x)** and press **Run Script**.

IMPORTANT The prime script takes approximately 12 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).

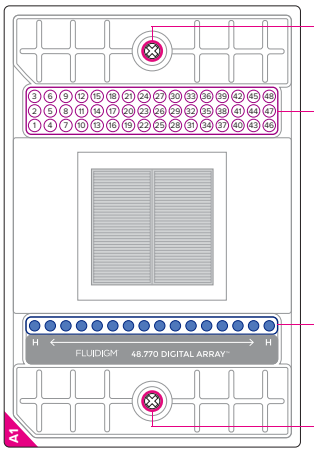


Figure 1. 48.770 IFC pipetting map

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

Instrument	Operation	Run Script	Continue to
Juno	Loading only	**Load 48.770**	For real-time dPCR: Collect Data For endpoint read: Thermal-Cycle the IFC (endpoint read only)
MX	Loading only	**Load (148x)**	
Juno (endpoint read only)	One-step loading and thermal-cycling	**One Step 48.770**	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 48.770 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 48.770**
FC1 cycler	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 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.

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**.
Probes	Select probe types. **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.

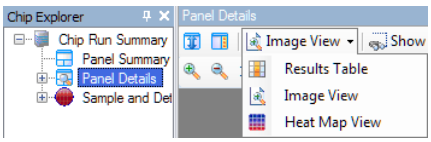
☐ **12** Click **Update** to see the changes reflected in the highlighted wells.

☐ **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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