Gene Expression with the 48.48 IFC Using Fast TaqMan Assays (Biomark HD Only)

For more information, see the Real-Time PCR Analysis User Guide (PN 68000088) and the Juno System User Guide (PN 100-7070).

Review Juno/IFC Controller MX Workflow

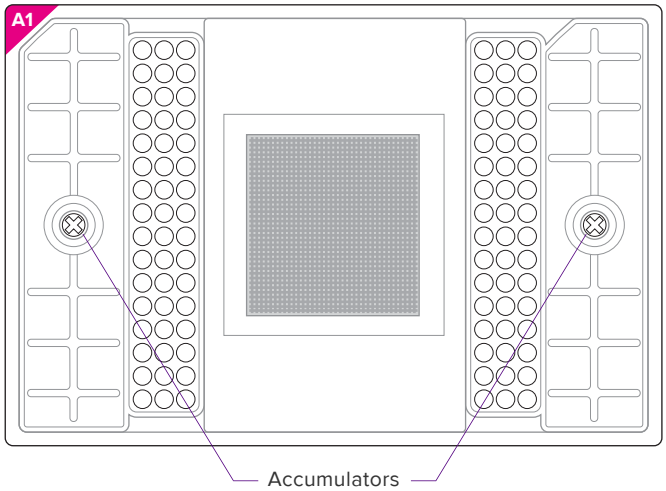
Prime	Load	Thermal-Cycle (PCR) and image
Juno™ or MX	Juno or MX	Biomark™ HD

Prime the 48.48 Dynamic Array IFC

IMPORTANT

- Use the 48.48 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening package.
- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of control line fluid.
- Control line fluid on IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

1. Inject control line fluid into each accumulator on the IFC.
2. Remove and discard the blue protective film from the bottom of the IFC.
3. Place the IFC into the instrument and run the prime script:
   - Juno: **Prime 48.48 GE**
   - MX: **Prime (113x)**



Prepare 10X Assays

In a DNA-free hood, prepare aliquots of 10X assays using volumes in the following table. Scale up appropriately for multiple runs.

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Vol. for 50 µL Stock
20X TaqMan® Gene Expression Assay (Life Technologies)	2.5	3.0	25.0
2X Assay Loading Reagent (Fluidigm PN 100-7611)	2.5	3.0	25.0
Total	**5.0**	**6.0**	**50.0**

Final concentration (at 10X): primers, 9 µM; probe, 2.5 µM

Prepare Sample Pre-Mix and Samples

1. Combine components in the following table to make sample pre-mix and final sample mixture. Scale up appropriately for multiple runs.

Component	Vol. Per Inlet (µL)	Vol. Per Inlet with Overage (µL)	Sample Pre-Mix for 48.48* (µL)
SAMPLE PRE-MIX			
2X master mix†	2.5	3.0	180.0
20X GE Sample Loading Reagent (Fluidigm PN 100-7610)	0.25	0.3	18.0
Preamplified cDNA‡	2.25	2.7	—
Total	**5.0**	**6.0**	**—**

*60 reactions for ease of pipetting

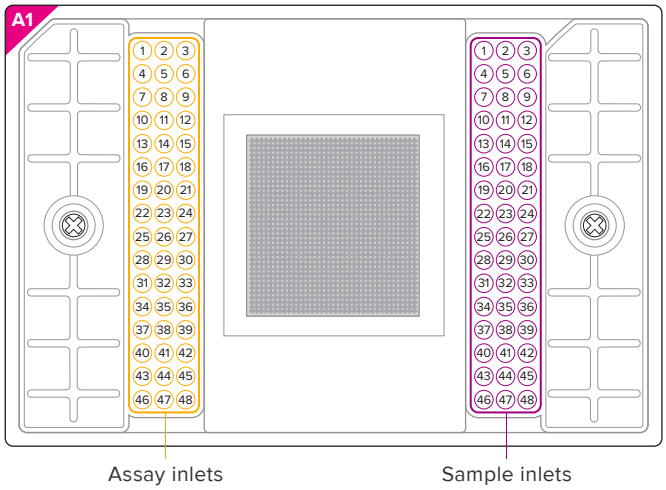
† Quanta PerfeCTa® qPCR Fast Mix®, Low ROX™ (Quanta Biosciences, PN 95078-012 or VWR, PN 1014190-220) or TaqMan Fast Universal PCR Master Mix (Life Technologies, PN 435042) or TaqMan GTXpress™ Master Mix (Life Technologies, PN 4401892) or TaqMan Fast Advanced Master Mix (Life Technologies, PN 4444557)

‡ For more information about PreAmp treatment, see Gene Expression PreAmp with Fluidigm PreAmp Master Mix and TaqMan Assays Quick Reference (PN 100-5876).

2. In a DNA-free hood, combine the master mix with the 20X GE Sample Loading Reagent in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Vortex to mix and centrifuge briefly. Aliquot 3.3 µL of this sample pre-mix for each sample.

3 Remove the aliquots of sample pre-mix from the DNA-free hood and in a DNA sample hood, add 2.7 µL of sample to each, making a total volume of 6 µL in each aliquot. Vortex to mix and centrifuge.

48.48 IFC Pipetting Map



Load the IFC

IMPORTANT

- Vortex thoroughly and centrifuge all assay and 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.
- For unused assay inlets, use 3.0 µL assay loading reagent and 3.0 µL water per inlet.
- For unused sample inlets, use 3.3 µL of sample mix and 2.7 µL of water per inlet.

1 When the prime script has finished, remove the primed IFC from the instrument and pipet 5 µL of each assay and each sample into their respective inlets on the IFC.

2 Return the IFC to the instrument and run the load script:

- Juno: **Load Mix 48.48 GE**
- MX: **Load Mix (113x)**

IMPORTANT Start IFC run within 1 hour of loading samples.

Collect Real-Time PCR Data

Biomark HD Data Collection software v3.0.2 or higher is required for collecting data.

1 Remove any dust particles or debris from the IFC surface.

2 Double-click the **Data Collection** icon on the desktop to launch the software.

3 Click **Start a New Run**.

4 Place the IFC into the instrument.

5 Click **Load**.

6 Verify IFC barcode and IFC type.

7 Choose project settings (if applicable). Click **Next**.

8 Provide a name and select a file storage location for a new IFC run, or browse to select a predefined run file. Click **Next**.

9 Choose the application, reference, and probes:

a Application type: **Gene Expression**

b Passive reference: **ROX**

c Assay: **Single probe**

d Probe type: **FAM-MGB**

e Click **Next**.

10 Browse to and choose a thermal protocol:
GE 48x48 Fast v1.pcl
Be sure to use a 48.48-specific protocol.

11 Confirm **Auto Exposure** is selected. Click **Next**.

12 Verify the IFC run information.

13 Click **Start Run**.

For technical support visit fluidigm.com/support

EMAIL **United States** techsupport@fluidigm.com | **Europe** techsupporteurope@fluidigm.com
Asia techsupportasia@fluidigm.com | **Latin America** techsupportlatam@fluidigm.com
All other countries techsupport@fluidigm.com

PHONE **United States (toll-free)** +1 866 358 4354 | **Europe** +33 1 60 92 42 40
Japan +81 3 3662 2150 | **China (excluding Hong Kong)** +86 21 3255 8368
All other countries +1 650 266 6100