Gene Expression with the 192.24 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).

NOTE Preamplification of samples is required. See Fluidigm Gene Expression Specific Target Amplification Quick Reference (PN 68000133) for more detail.

Review Juno/IFC Controller RX Workflow

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

Prepare the 192.24 IFC

IMPORTANT

- Use the 192.24 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening the package.
- Due to different accumulator volumes, use only syringes with 150 µL of control line fluid.
- Control line fluid on the IFC or in the inlets makes the IFC unusable.

1 Inject control line fluid into accumulator 2 (Acc2) on the IFC.

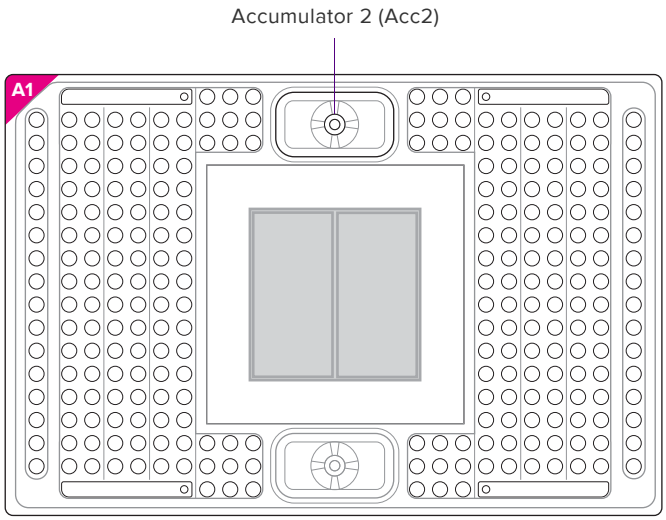
2 Remove and discard the blue protective film from the bottom of the IFC.

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)	1.5	2.0	25.0
2X Assay Loading Reagent (Fluidigm PN 100-7611)	1.5	2.0	25.0
Total	**3.0**	**4.0**	**50.0**

Final concentration (at 10X): primers, 9 µM; probe, 2 µM



Prepare Sample Pre-Mix and Samples

1 Combine components in table below 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 192.24 with overage* (µL)
SAMPLE PRE-MIX			
2X master mix†	1.5	2.0	480.0
20X GE Sample Loading Reagent (Fluidigm PN 100-7610)	0.15	0.2	48.0
Preamplified‡ cDNA	1.35	1.8	—
Total	**3.0**	**4.0**	**—**

*240 reactions for ease of pipetting.

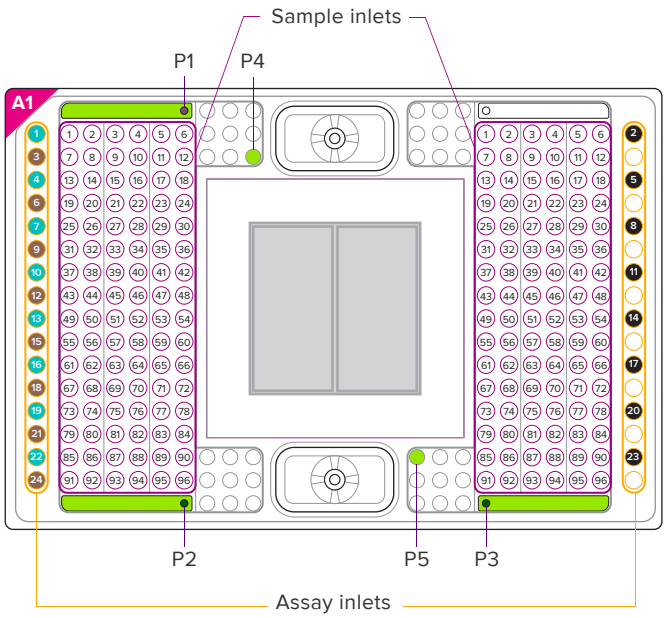
† Quanta PerfeCTa® qPCR Fast Mix®, Low ROX™ (Quanta Biosciences PN 95078-012 or VWR, PN 1014190-220) or TaqMan Fast Universal PCR Master Mix (Life Technologies PN 4352042) or TaqMan GTXpress™ Master Mix (Life Technologies PN 4401892) or TaqMan Fast Advanced Master Mix (Life Technologies PN 4444557)

‡ 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 2X master mix with the GE sample loading reagent in a 1.5 mL sterile tube—enough volume to fill an entire IFC. 2.2 µL of this sample pre-mix can then be aliquoted for each sample.

3 Remove these aliquots from the DNA-free hood and add 1.8 µL of cDNA to each, making a total volume of 4 µL in each aliquot.

192.24 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 2.0 µL assay loading reagent and 2.0 µL of water per inlet.
- For unused sample inlets, use 2.2 µL of sample pre-mix and 1.8 µL of water per inlet.

1 Pipet 3 µL of each assay and 3 µL of each sample into the respective inlets on the IFC (see the 192.24 IFC pipetting map).

2 Pipet 150 µL of Actuation Fluid into the P1 well on the IFC.

3 Pipet 150 µL of Pressure Fluid into the P2 and P3 wells on the IFC.

4 Pipet 20 µL of Pressure Fluid into the P4 and P5 wells on the IFC.

5 Blot the IFC surface with a dry, lint-free cloth.

6 Place the IFC into the Juno or RX, then run the script:

- Juno: **Load Mix 192.24 GE**
- RX: **Load Mix (169x)**

IMPORTANT Start IFC run within 1 hour of loading samples.

Collect Real-Time PCR Data

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

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

3 Click **Start a New Run**.

4 Place the loaded IFC into the Biomark HD.

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

6 Click **Load**.

7 Verify IFC barcode and IFC type.

a Choose project settings (if applicable)

b 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 the thermal protocol: **GE 192x24 Fast v1.pcl**

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

12 Verify 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