

Gene Expression with the 96.96 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 HX Workflow

| Prime | Load | Thermal-Cycle (PCR) and Image |
|-------------|------------|-------------------------------|
| Juno™ or HX | Juno or HX | Biomark™ HD |

Prime the 96.96 IFC

IMPORTANT

- Use the 96.96 Dynamic Array™ integrated fluidic circuit (IFC) within 24 hours of opening package.
- Due to different accumulator volumes, only use 96.96 syringes with 150 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.

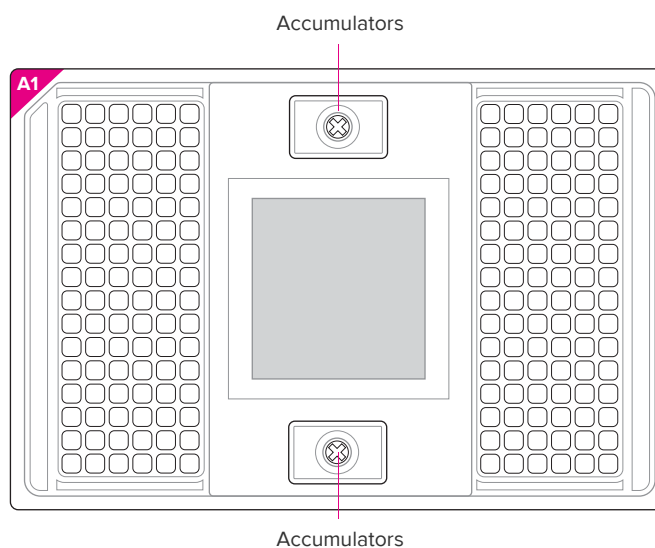
- 1 Inject control line fluid into each accumulator on the IFC.
- 2 Remove and discard the blue protective film from the bottom of the IFC.
- 3 Place the IFC into the instrument and run the prime script:
 - Juno: **Prime 96.96 GE**
 - HX: **Prime (136x)**

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 | 25 |
| 2X Assay Loading Reagent (Fluidigm PN 100-7611) ● | 2.5 | 3 | 25 |
| Total | 5.0 | 6 | 50 |

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 96.96* (µL) |
|--|---------------------|----------------------------------|--------------------------------|
| SAMPLE PRE-MIX | | | |
| 2X Master Mix† | 2.5 | 3.0 | 360.0 |
| 20X GE Sample Loading Reagent (Fluidigm PN 100-7610) ● | 0.25 | 0.3 | 36.0 |
| Preamplified cDNA‡ | 2.25 | 2.7 | — |
| Total | 5.0 | 6.0 | — |

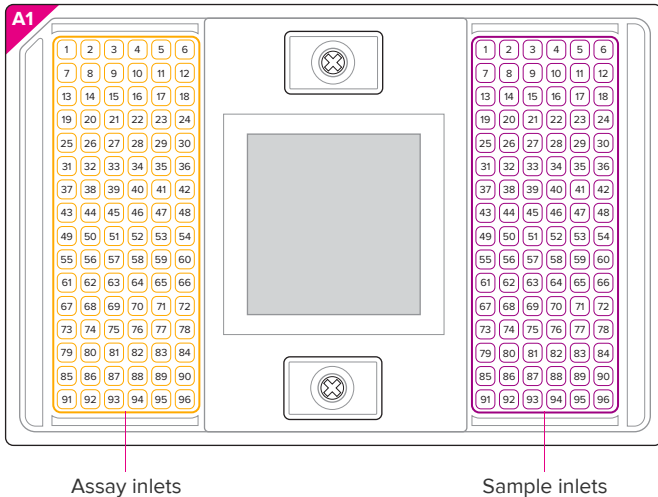
* 120 reactions for ease of pipetting

† 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.

96.96 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 μ L assay loading reagent and 3.0 μ L water.
- For unused sample inlets, use 3.3 μ L of sample mix and 2.7 μ L of DNA-free water per inlet.

- 1 When the prime script has finished, remove the primed IFC from the instrument and pipet 5 μ L 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 96.96 GE**
 - HX: **Load Mix (136x)**

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 96x96 Fast v2.pcl
Be sure to use a 96.96-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.

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