

Gene Expression with the 96.96 IFC Using Delta Gene Assays on Preamplified Samples

To preamplify samples, refer to the Preamplification of cDNA for Gene Expression with Delta Gene Assays Quick Reference (PN 100-5875). For more information on Juno use, refer to the Juno System User Guide (PN 100-7070). For more information on collecting real-time data, refer to the Real-Time PCR Analysis User Guide (PN 68000088).

Review Juno/IFC Controller HX Workflow

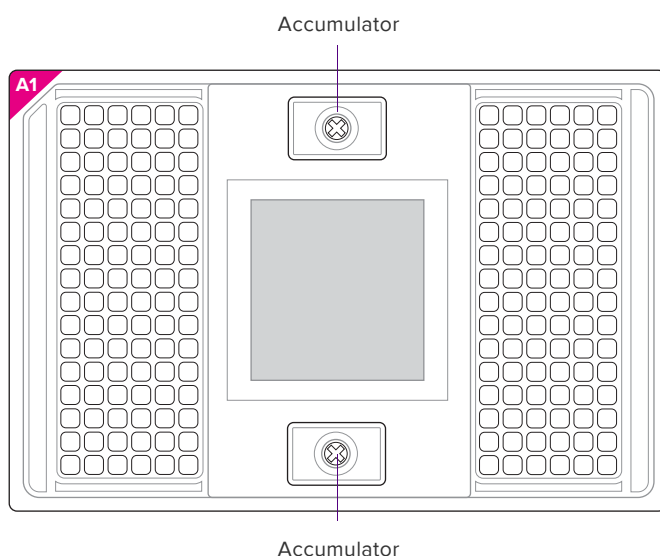
Prime	Load	Thermal-Cycle (PCR) and image
Juno™ or HX	Juno or HX	Biomark™ HD or Biomark

Prime the 96.96 IFC

! IMPORTANT

- Use the 96.96 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 film on 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 Sample Pre-Mix and Samples

- 1 **! IMPORTANT** Vortex thoroughly and centrifuge all assay and sample solutions before pipetting into IFC inlets. Failure to do so may result in a decrease in data quality. Avoid bubbles.
- 1 In a new microcentrifuge tube, combine the SsoFast™ EvaGreen® Supermix with the 20X DNA Binding Dye Sample Loading Reagent. Vortex and centrifuge the sample pre-mix at 1,000 x g for 10 seconds (see Table 1).
- 2 Aliquot 3.3 μL of pre-mix to each well of a 96-well plate. Next, add 2.7 μL of preamplified and Exo I-treated sample to individual wells.
- 3 Vortex the samples for ≥ 20 seconds, then centrifuge the samples at prior to adding the samples at 1,000 x g for ≥ 30 seconds. to the IFC. You can store the samples at 4 °C for ≤ 1 day.

Table 1: Sample mix

Component	Vol. per Inlet (μL)	Vol. per Inlet with overage (μL)	Vol. for 96.96 IFC overage (μL ; 60 samples)
SAMPLE PRE-MIX			
2X SsoFast EvaGreen Supermix with low ROX (BioRad PN 172-5211)	2.5	3.0	360.0
20X DNA Binding Dye (Fluidigm PN 100-7609)	0.25	0.3	36.0
Preamplified and Exo 1-treated sample	2.25	2.7	—
Total	5.0	6.0	—

Prepare the Assay Mix

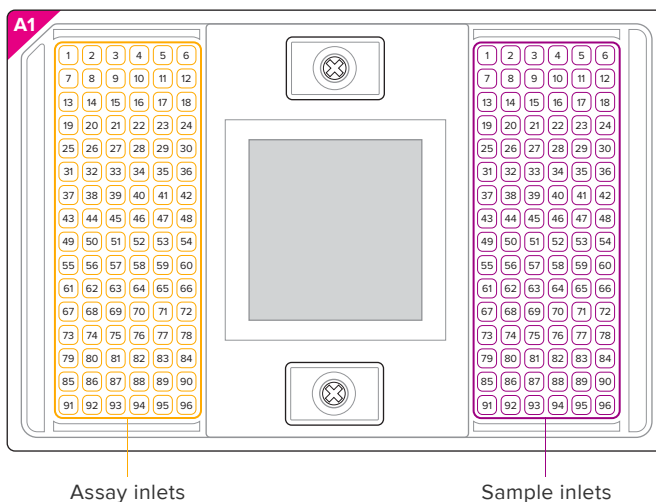
- 1 In a new microcentrifuge tube, dilute 100 μM combined forward and reverse primers:

Component	Vol. per Inlet (μL)	Vol. per Inlet with Overage (μL)	Vol. for 50- μL Stock (μL)
2X Assay Loading Reagent (Fluidigm PN 100-7611)	2.5	3.0	25
1X DNA Suspension Buffer (TEKnova PN T0221)	2.25	2.7	22.5
100 μM combined forward and reverse primers	0.25	0.3	2.5
Total*	5.0	6.0	50.0

*The final concentration of each primer is 5 μM in the inlet and 500 nM in the final reaction.

- 2 Vortex the assay mix for ≥ 20 seconds, and then centrifuge the samples at prior to adding the samples at 1,000 $\times g$ for ≥ 30 seconds before pipetting the assays into the IFC inlets.

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.

! IMPORTANT Do not leave any inlets empty.

- For unused assay inlets, use 3.0 μL assay loading reagent and 3.0 μL water.
- For unused sample inlets, use 3.3 μL of sample mix and 2.7 μL of DNA-free 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 5 μL of 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 the IFC run within 1 hour of loading the samples.

Collect Real-Time PCR Data

- 1 Remove any dust particles or debris from the IFC surface.
- 2 Double-click the **Data Collection** icon on the desktop.
- 3 Click **Start a New Run**.
- 4 Ensure that the status indicators for the lamp (Biomark only) and the camera are green.
- 5 Place the IFC into the instrument, and then 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 Choose assay: **Single probe**.
 - d Select probe types: **EvaGreen** and then click **Next**.
- 10 Browse to and choose the appropriate thermal protocol:
 - For Biomark HD: **GE Fast 96x96 PCR+Melt v2.pcl**
 - For BioMark: **GE 96x96 PCR+Melt v2.pcl**
- ! **IMPORTANT** Be sure to use a 96.96-specific protocol.
- 11 Confirm **Auto Exposure** is selected. Click **Next**.
- 12 Verify the IFC run information, and then click **Start Run**.

For technical support visit fluidigm.com/support

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