Gene Expression with the 96.96 IFC Using Standard TaqMan Assays

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 or Biomark

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 the IFC or in the inlets makes the IFC unusable.
- Load the IFC within 60 minutes of priming.

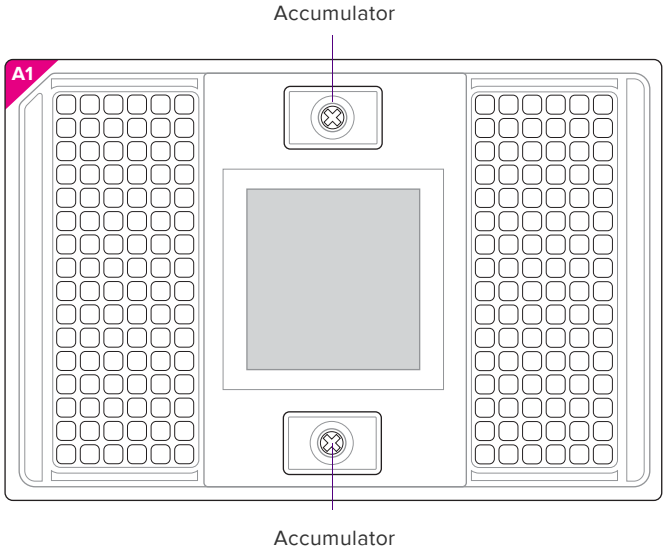
1. Inject control line fluid into each accumulator on the IFC.
2. Remove and discard the blue protective film from the bottom of the IFC.
3. Place the IFC into the Juno or HX, then run the script:
   - Juno: **Prime 96.96 GE**
   - HX: **Prime (136x)**

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 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.

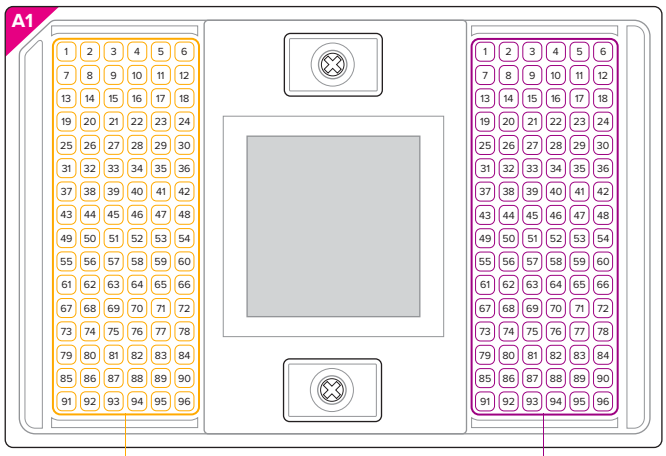
Component	Vol. per inlet (µL)	Vol. per inlet with overage (µL)	Sample pre-mix for 96.96* (µL)
SAMPLE PRE-MIX			
TaqMan Universal PCR Master Mix (2X) (Life Technologies PN 4304437)	2.5	3.0	360.0
20X GE Sample Loading Reagent (Fluidigm PN 100-7610)	0.25	0.3	36.0
Preamplified cDNA†	2.25	2.7	—
Total	**5.0**	**6.0**	**—**

*120 reactions for ease of pipetting

† 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 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 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

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 Ensure that the status indicators for the lamp (Biomark only) and the camera are green.

5 Place the IFC into the instrument.

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

7 Click **Load**.

8 Verify IFC barcode and IFC type.

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

10 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**.

f Browse to and choose a thermal protocol:
GE 96x96 Standard v1.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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