

# Gene Expression with the 48.48 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 MX Workflow

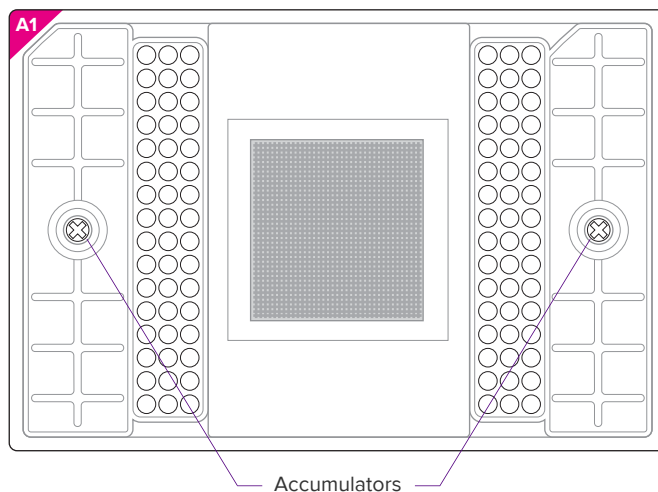
Prime	Load	Thermal-cycle (PCR) and image
Juno™ or MX	Juno or MX	Biomark™ HD or Biomark

## Prime the 48.48 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 film on 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
<b>Total</b>	<b>5.0</b>	<b>6.0</b>	<b>50.0</b>

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.

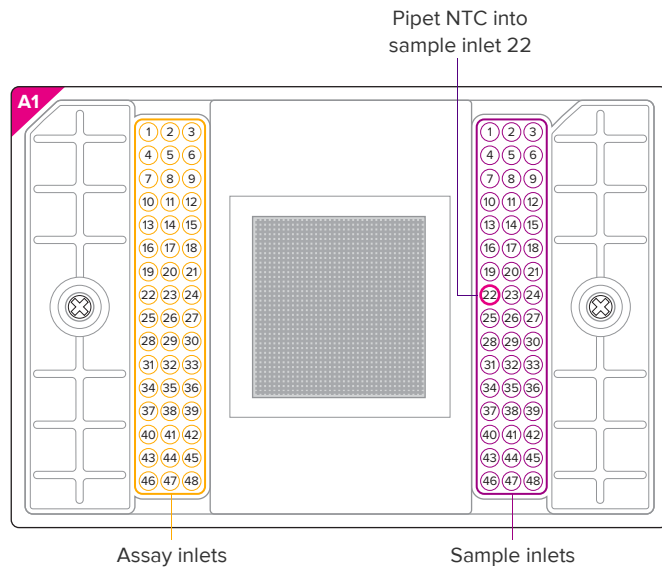
Component	Vol. per inlet (µL)	Vol. per inlet with overage (µL)	Sample pre-mix for 48.48* (µL)
<b>SAMPLE PRE-MIX</b>			
TaqMan Universal PCR Master Mix (2X) (Life Technologies PN 4304437)	2.5	3.0	180
20X GE Sample Loading Reagent (Fluidigm PN 100-7610) ●	0.25	0.3	18
Preamplified cDNA†	2.25	2.7	—
<b>Total</b>	<b>5.0</b>	<b>6.0</b>	<b>—</b>

\*60 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 TaqMan Universal PCR Master Mix with the GE Sample Loading Reagent in a 1.5 mL sterile tube—enough volume to fill an entire IFC. Aliquot 3.3 µL of this sample pre-mix for each sample.
- 3 Remove the aliquots from the DNA-free hood and add 2.7 µL of cDNA to each, making a total volume of 6 µL in each aliquot.

## 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.
- Do not leave any inlets empty.  
For unused assay inlets, use 3.0  $\mu\text{L}$  assay loading reagent and 3.0  $\mu\text{L}$  water per inlet.  
For unused sample inlets, use 3.3  $\mu\text{L}$  of sample mix and 2.7  $\mu\text{L}$  of water per inlet.
- Run NTC in sample inlet 22.

- 1 When the prime script has finished, remove the primed IFC from the instrument and pipet 5  $\mu\text{L}$  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 48.48 GE**
  - MX: **Load Mix (113x)**

**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 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 Click **Load**.
- 7 Verify IFC barcode and IFC type.
- 8 Choose project settings (if applicable). Click **Next**.
- 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**.
- 11 Browse to and choose thermal protocol: **GE 48x48 Standard v1.pcl**  
Be sure to use a 48.48-specific protocol.
- 12 Confirm **Auto Exposure** is selected. Click **Next**.
- 13 Verify the IFC run information.
- 14 Click **Start Run**.

### For technical support visit [fluidigm.com/support](https://fluidigm.com/support)

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