# Gene Expression with the 192.24 IFC Using Delta Gene 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 RX Workflow

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

## 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.

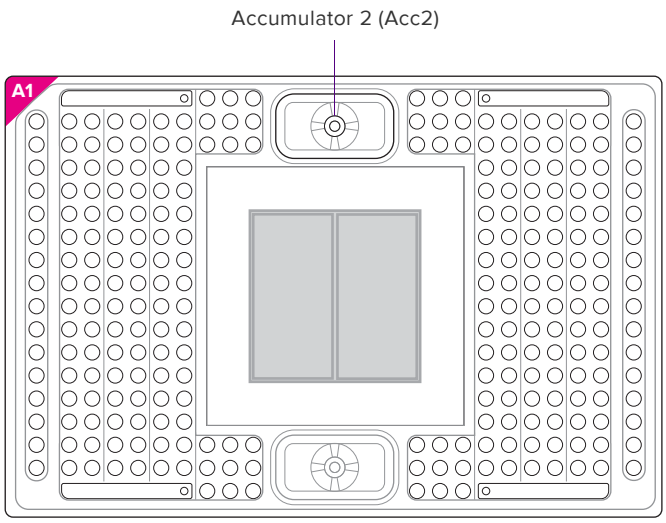
1 Inject control line fluid into accumulator 2 (Acc2) on the IFC.

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

## Prepare 10X Assays

1 Prepare a 10X assay mix. Scale up appropriately for multiple runs.

| Assay mix component | Vol. per Inlet (µL) | Vol. per inlet with overage (µL) | Vol. for 30 µL stock |
|---|---|---|---|
| 100 µM each Delta Gene™ primers (forward and reverse combined), non-wet-lab tested (ASY-GE) or wet-lab tested (ASY-GE WET) | 0.15 | 0.2 | 1.5 |
| 1X DNA suspension buffer | 1.35 | 1.8 | 13.5 |
| 2X Assay Loading Reagent (PN 100-7611) | 1.5 | 2.0 | 15.0 |
| **Total** | **3.0** | **4.0** | **30.0** |




## Prepare Sample Pre-Mix and Samples

**IMPORTANT** Failure to do the following may result in a decrease in data quality.

- Pipet with care. The Delta Gene Sample Reagent is extremely viscous. **Do not vortex the Delta Gene Sample Reagent by itself at its stock concentration.**
- Vortex thoroughly and centrifuge all assay and sample solutions **except** the 192.24 Delta Gene Sample Reagent before pipetting into IFC inlets. You can thaw the 192.24 Delta Gene Sample Reagent up to two times only.

1 Combine the SsoFast™ EvaGreen® Supermix with the 192.24 Delta Gene Sample Reagent. Vortex and spin down the sample pre-mix.

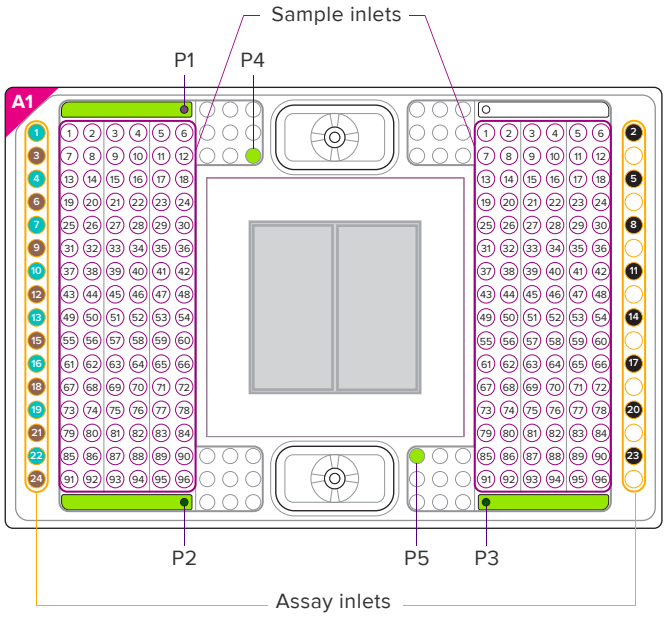
2 Aliquot 2.2 µL to each well of a 96-well plate. Next, add 1.8 µL of sample to individual wells. To remove any bubbles, vortex samples and centrifuge prior to adding the samples to the IFC.

| Component | Vol. per inlet (µL) | Vol. per inlet with overage (µL) | Sample pre-mix with overage (µL)* |
|---|---|---|---|
| SAMPLE PRE-MIX | | | |
| 2X SsoFast EvaGreen Supermix with low ROX™ (Bio-Rad PN 172-5211) | 1.5 | 2.0 | 420.0 |
| 192.24 Delta Gene Sample Reagent (PN 100-6653) | 0.15 | 0.2 | 42.0 |
| Preamplified and Exo I-treated cDNA† | 1.35 | 1.8 | — |
| **Total** | **3.0** | **4.0** | **—** |

*For more information about PreAmp and Exonuclease I treatment, see Gene Expression PreAmp with Fluidigm PreAmp Master Mix and TaqMan Assays Quick Reference (PN 100-5875).

†For more information about PreAmp treatment, see Preamplification of cDNA for Gene Expression with Delta Gene Assays Quick Reference (PN 100-5875).

## 192.24 IFC Pipetting Map




## Load the IFC

**IMPORTANT**

- 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 (PN 100-6250) into the P1 well on the IFC.

3 Pipet 150 µL of Pressure Fluid (PN 100-6249) into the P2 and P3 wells on the IFC.

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

5 Place the IFC into the instrument and run the load 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.

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

2 Select **Start a New Run**.

3 Verify that the camera and/or lamp are ready.

4 Confirm that the blue tape has been removed from the bottom of the IFC.

5 Select **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: **EvaGreen**

e Click **Next**.

10 Select thermal cycling protocol:

- Biomark HD only (fast): **GE 192x24 Fast PCR+Melt v2.pcl**
- Biomark HD or Biomark (standard): **GE 192x24 PCR+Melt v2.pcl**

11 Confirm **Auto Exposure** is selected.

12 Click **Next**.

13 Verify IFC run information.

14 Select **Start Run**.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