Gene Expression with the Flex Six 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 HX Workflow

Prime	Load	Thermal-cycle (PCR) and image	Post-run
Juno™ or HX	Juno or HX	Biomark™ HD or Biomark	Juno or HX

Prime the Flex Six IFC (first use only)

Once the IFC is primed, skip these steps on subsequent use.

IMPORTANT

- Use the Flex Six™ integrated fluidic circuit (IFC) within three months of opening the package.
- Control line fluid on IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

1. Using the included syringes, inject 150 µL of control line fluid into each accumulator. Do not remove the barrier plugs until you load the IFC.
2. Remove and discard blue protective film from bottom of IFC.
3. Place the IFC into the instrument and run the prime script:
   - Juno: **Prime Flex Six GE**
   - HX: **Prime (153x)**

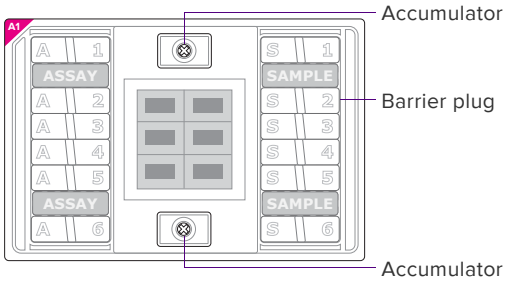
Prepare 10X Assays

We recommend preparing 10X assay stock, due to the small pipetting volumes needed to prepare a single assay mix. Unused 10X assays can be stored at –20 °C for up to three weeks.

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./inlet with overage (µL)	Vol. for 40 µL stock* (µL)
100 µM each Delta Gene™ primers (forward and reverse combined; 100 µM each), non-wet-lab tested (ASY-GE) or wet-lab tested (ASY-GE WET)	0.15	0.2	2.0
DNA suspension buffer	1.35	1.8	18.0
2X Assay Loading Reagent (Fluidigm PN 100-5359)	1.5	2.0	20.0
Total	**3.0**	**4.0**	**40.0**

*Enough for 10 replicates



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 Flex Six Delta Gene Sample Reagent before pipetting into IFC inlets. You can thaw the Flex Six Delta Gene Sample Reagent up to six times only.

Combine components in the table below to make the sample pre-mix and final sample mixture in a 96-well plate, tubes, or tube strips. Scale up appropriately for multiple runs.

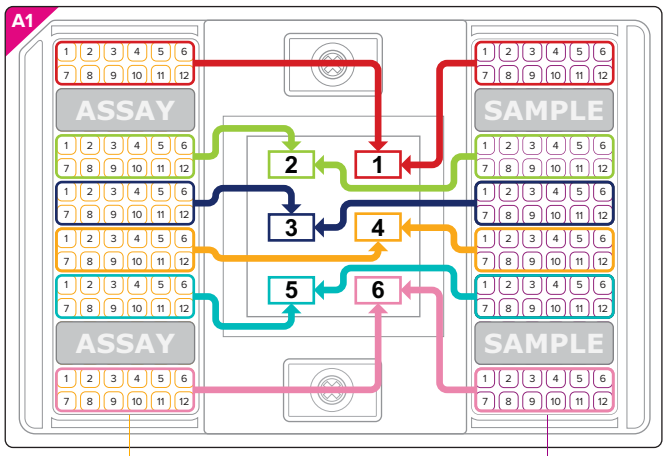
1. In a DNA-free hood, combine the Sample Pre-Mix components to make enough for your experiment (33 µL/partition). Aliquot 2.2 µL of the pre-mix for each sample.
2. Remove the aliquots from the DNA-free hood and add 1.8 µL of PreAmp and Exo I-treated sample to each, making a total volume of 4 µL in each aliquot. Vortex and spin down the final sample mixture.

Component	Vol. per inlet (µL)	Vol./inlet with overage (µL)	Sample pre-mix for 1 partition* (µL)
SAMPLE PRE-MIX			
SsoFast™ EvaGreen® Supermix with Low ROX™ (2X) (Bio-Rad PN 1772-5211)	1.50	2.0	30.0
Flex Six Delta Gene Sample Reagent (Fluidigm PN 100-7673)	0.15	0.2	3.0
PreAmp and Exo I-treated sample† (added individually to sample pre-mix)	1.35	1.8	—
Total	**3.00**	**4.0**	**—**

*15 reactions for ease of pipetting

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

Flex Six Partitions and Inlets



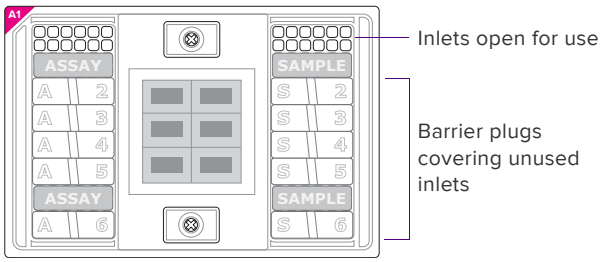
Load the IFC

Each Flex Six IFC has a total of six independent partitions (1–6 above). Each partition has a 12 × 12 format (12 assay inlets and 12 sample inlets) and can be run independently as a separate experimental run (at different times or on different days) or simultaneously.

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.
- At minimum, all 12 assay inlets and all 12 sample inlets for a partition must be filled.
  For unused assay inlets in active partitions, prepare 2.0 µL assay loading reagent and 2.0 µL water per inlet.
  For unused sample inlets in active partitions, prepare 2.2 µL sample pre-mix and 1.8 µL water per inlet.

1 Be sure barrier plugs are placed on unused inlets to prevent pipetting into the wrong inlets and to track used/unused partitions.



2 Pipet one partition at a time by removing the barrier plugs for that particular partition.

3 Pipet 3 µL of each assay and each sample into their respective inlets. Do not replace the barrier plugs after pipetting.

4 Return the IFC to the instrument and run the load script:

- Juno: **Load Mix Flex Six GE**
- HX: **Load Mix (153x)**

Do not replace barrier plugs after loading.

Collect Real-Time PCR Data

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

IMPORTANT If this is your first time running a Flex Six IFC, set up a tracking file: select **Tools** > **FLEXsix Usage Tracking**. Click **New**, enter a filename, and select a location. Click **Done**.

2 Click **Start a New Run**.

3 Remove debris from the top of the IFC with clear tape.

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

5 Place the loaded IFC into Biomark HD or Biomark.

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

7 Click **Load**.

8 Select the partitions you wish to run.

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 Browse to and choose a thermal protocol:

- Biomark (Standard): **GE Flex Six PCR+Melt v1**
- Biomark HD (Fast): **GE Flex Six Fast PCR+Melt v1**

11 Confirm **Auto Exposure** is selected.

12 Click **Start Run**.

Perform Post-Run

1 Immediately after the IFC run, return the IFC to Juno or HX and run the post-run script to relax the valves:

- Juno: **Post Run Flex Six GE**
- HX: **Post Run (153x)**

2 Put the barrier plugs back into the used inlets. Label used barrier plugs to record which partitions/inlets were used.

3 Store the IFC at room temperature and protect from dust.

For technical support visit fluidigm.com/support

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