

# Gene Expression with the Flex Six IFC Using Fast/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	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.
- Using the included syringes, inject 150  $\mu\text{L}$  of control line fluid into each accumulator. Do not remove the barrier plugs until you load the IFC.
  - Remove and discard blue protective film from bottom of IFC.
  - Place the IFC into the instrument and run the prime script:
    - Juno: **Prime Flex Six GE**
    - HX: **Prime (153x)**

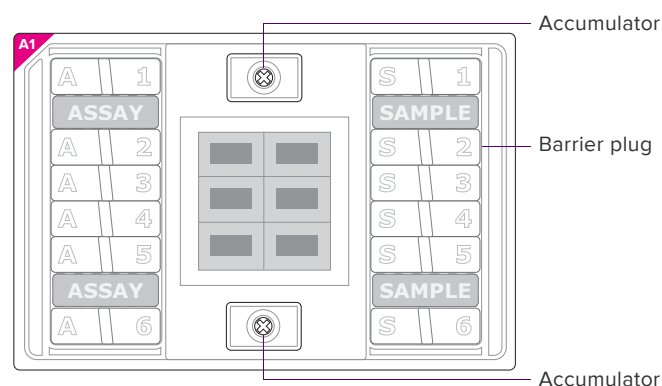
## Prepare 10X Assays

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  $-20\text{ }^{\circ}\text{C}$  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 ( $\mu\text{L}$ )	Vol. per inlet with overage ( $\mu\text{L}$ )	Vol. for 40 $\mu\text{L}$ stock ( $\mu\text{L}$ )*
TaqMan® Gene Expression Assay (20X) (Life Technologies)	1.5	2.0	20.0
2X Assay Loading Reagent (Fluidigm PN 100-5359) ●	1.5	2.0	20.0
<b>Total</b>	<b>3.0</b>	<b>4.0</b>	<b>40.0</b>

\*10 replicates



## Prepare Sample Pre-Mix and Samples

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.

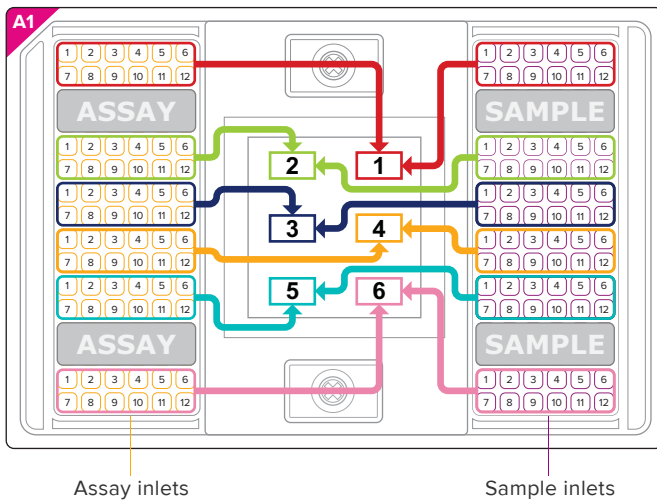
- In a DNA-free hood, combine the sample pre-mix components to make enough for your experiment (33  $\mu\text{L}$ /partition). Aliquot 2.2  $\mu\text{L}$  of the pre-mix for each sample.
- Remove the aliquots from the DNA-free hood and add 1.8  $\mu\text{L}$  of preamplified cDNA to each, making a total volume of 4  $\mu\text{L}$  in each aliquot.

Component	Vol. per Inlet ( $\mu\text{L}$ )	Vol. per inlet with overage ( $\mu\text{L}$ )	Sample pre-mix for 1 partition ( $\mu\text{L}$ )*
<b>SAMPLE PRE-MIX</b>			
<b>Fast:</b> TaqMan Fast Advanced Master Mix (2X) (Life Technologies, PN 4444557)	1.50	2.0	30.0
OR			
<b>Standard:</b> TaqMan Gene Expression PCR Master Mix (2X) (Life Technologies PN 4369016)			
20X GE Sample Reagent (Fluidigm PN 100-6311) ●	0.15	0.2	3.0
Preamplified cDNA <sup>†</sup> (added individually to sample pre-mix)	1.35	1.8	—
<b>Total</b>	<b>3.00</b>	<b>4.0</b>	<b>—</b>

\*15 reactions for ease of pipetting

<sup>†</sup>For more information about PreAmp and Exonuclease I treatment, see Gene Expression PreAmp with Fluidigm PreAmp Master Mix and TaqMan Assays Quick Reference (PN 100-5876).

## Flex Six Partitions and Inlets



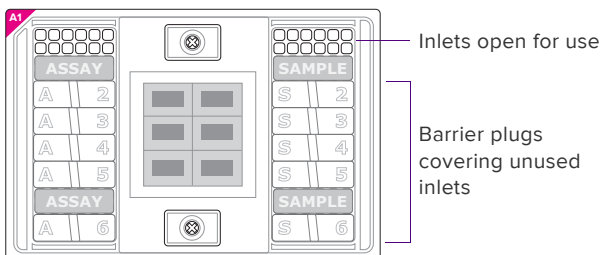
### Load the IFC

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  $\mu\text{L}$  assay loading reagent and 2.0  $\mu\text{L}$  water per inlet.  
For unused sample inlets in active partitions, prepare 2.2  $\mu\text{L}$  sample pre-mix and 1.8  $\mu\text{L}$  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.

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

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- 3 Pipet 3  $\mu\text{L}$  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.
  - a Application type: **Gene Expression**
  - b Passive reference: **ROX**
  - c Assay: **Single probe**
  - d Probe type: **FAM-MGB**
  - e Click **Next**.
- 9 Browse to and choose a thermal protocol:
  - Biomark HD only (fast): **GE FLEXSix Fast v2**
  - Biomark HD or Biomark (standard): **GE FLEXSix Standard v1**
- 10 Confirm **Auto Exposure** is selected.
- 11 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.