Digital PCR with the 12.765 IFC Using Gene-Specific Assays

For more information, see the Digital PCR Analysis User Guide (PN 68000100) and the Juno System User Guide (PN 100-7070).

Choose a Juno/IFC Controller MX Workflow

Prime	Load and thermal-cycle (PCR)	Image
Juno™	Juno one-step loading and PCR	Biomark™ HD or Biomark or EP1™

Prime	Load	Thermal-cycle (PCR)	Image
Juno™ or MX	Juno or MX	Juno or FC1™ cycler or Biomark HD/Biomark	Biomark™ HD or Biomark or EP1™

Prime the 12.765 Digital Array IFC

IMPORTANT

- Use the 12.765 Digital 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 protective film from bottom of IFC.
3. Place the IFC into the instrument and run the prime script:
   - Juno: **Prime 12.765**
   - MX: **Prime (115x)**

Prepare Sample Pre-Mix and Samples

1. Combine the components in the following table to make the sample pre-mix and the final sample mixture. Scale up appropriately for multiple runs.

Component	Vol./inlet (µL)	Vol./inlet with overage (µL)	Vol./IFC* (µL)
SAMPLE PRE-MIX			
TaqMan® Gene Expression Master Mix (Life Technologies PN 4369016)†	4.0	5.0	65.0
20X GE Sample Loading Reagent (Fluidigm PN 85000746) ●	0.4	0.5	6.5
20X gene-specific assays	0.4	0.5	6.5‡
DNA-free water	2.4	3.0	39.0
DNA	0.8	1.0	—
Total	**8.0**	**10.0**	**117.0**

*Enough for 13 reactions

†TaqMan Universal PCR Master Mix (Life Technologies, PN 4304437) may be substituted. Fluidigm recommends using TaqMan Gene Expression Master Mix for the Digital Array IFC.

‡The 20X assay can be removed from the sample pre-mix and added separately if different assays are to be used on the same IFC.

2. In a DNA-free hood, combine the TaqMan Gene Expression Master Mix, GE Sample Loading Reagent, DNA-free water, and 20X assay in a 1.5 mL sterile tube—enough volume to fill the entire IFC. 9.0 µL of this sample pre-mix can then be aliquoted for each sample (12 total).
3. Remove these aliquots from the DNA-free hood and add 1.0 µL of DNA to each, making a total volume of 10 µL in each aliquot.

Load the IFC

IMPORTANT

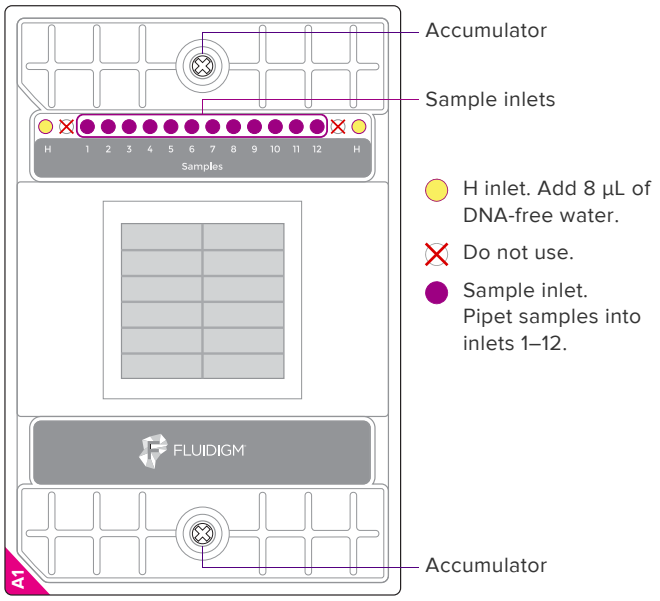
- Vortex thoroughly and centrifuge all sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

1. When the prime script has finished, remove the primed IFC from the instrument.
2. Pipet 8 µL of DNA-free water into each H inlet.
3. Pipet 8 µL sample mix into the sample inlets on the IFC.
4. Return the IFC to Juno or MX, then run the script according to operation:

Instrument	Operation	Run script	Continue to
Juno	One-step loading and PCR	**One Step 12.765**	"Collect End-Point Data"
Juno	Loading only	**Load 12.765**	"Thermal-Cycle the 12.765 IFC"
MX	Loading only	**Load (115x)**	"Thermal-Cycle the 12.765 IFC"

IMPORTANT Start IFC run within 1 hour of loading samples.

12.765 IFC Pipetting Map



Thermal-Cycle the 12.765 IFC

Choose the instrument and run the script:

Instrument	Operation	Run script
Juno	One-step loading and PCR	—
Juno	PCR only	**PCR 12.765**
Biomark HD or Biomark	PCR and imaging	Continue to "Collect End-Point Data" and select **dPCR Standard v1.pcl**

To run this protocol as an end-point read using the FC1 cycler or the Fluidigm stand-alone thermal cycler, see the FC1 Cycler Usage Quick Reference (PN 100-1250) or the Stand-alone Thermal Cycler Usage Quick Reference (PN 68000111), respectively.

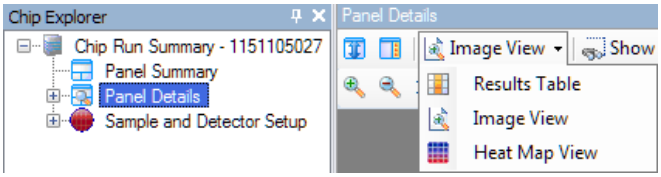
Collect End-Point Data

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 and EP1 only) and the camera are green.
5. Place the loaded IFC into the instrument. Click **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: **Digital PCR**.
   b. Passive reference: **ROX**.
   c. Assay: **Single probe**, **Two probes**, or **More than two probes**.
   d. Select probe types. Click **Next**.
10. Browse to and choose the thermal protocol: **dPCR Standard v1.pcl**
11. Confirm **Auto Exposure** is selected. Click **Next**.
12. Verify the IFC run information.
13. Click **Start Run**.

Use the Digital PCR Analysis Software

Be sure to click **Analyze** each time you change a parameter in the software.

1. Double-click the **Digital PCR Analysis** icon on the desktop to launch the software.
2. Click **Open a Chip Run**.
3. Double-click a **chiprun.bml** file to open it in the software.
4. Click **Sample and Detector Setup** in the Chip Explorer pane.
5. Click **New** or **Import**.
6. Highlight the wells and then annotate them.
7. Click **Editor** in the Sample and Detector Setup pane.
8. Choose **Sample Type** from the drop-down menu.
9. Enter a name for the sample.
10. Choose **Detector Type** from the drop-down menu.
11. Enter a name for the detector.
12. Click **Update** to see the changes reflected in the highlighted wells.
13. Click **Panel Summary** in the Chip Explorer pane.
14. Click **Analyze** in the Task pane.
15. Click **Panel Summary** or **Panel Details**.
16. Choose a view from the drop-down menu:
    - Results Table
    - Image View
    - Heat Map View



For technical support visit fluidigm.com/support

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