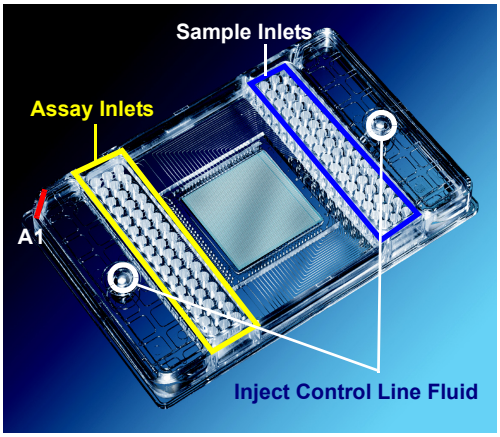
Fluidigm® 48.48 Real-Time PCR Workflow Quick Reference

PN 68000089, Rev. E

For more information see the *BioMark Real-Time PCR Analysis Software User Guide, PN 68000088*

1 Priming the 48.48 Dynamic Array™ IFC



CAUTION! USE THE 48.48 CHIP WITHIN 24 HOURS OF OPENING THE PACKAGE.

- DUE TO DIFFERENT ACCUMULATOR VOLUMES, ONLY USE 48.48 SYRINGES WITH 300 µL OF CONTROL LINE FLUID.
- CONTROL LINE FLUID ON THE CHIP OR IN THE INLETS MAKES THE CHIP UNUSABLE.
- LOAD THE CHIP WITHIN 60 MINUTES OF PRIMING.

1 Inject control line fluid into each accumulator on the chip.

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

3 Place the chip into the IFC (Integrated Fluidic Circuit) Controller MX, then run the **Prime (113x)** script to prime the control line fluid into the chip.

2 Preparing 10X Assays

1 In a DNA-free hood, prepare aliquots of 10X assays using volumes in table below (scale up appropriately for multiple runs).

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Volume for 50 µL Stock
20X TaqMan® Gene Expression Assay (Applied Biosystems)	2.5	3	25
2X Assay Loading Reagent (Fluidigm, PN 85000736) ●	2.5	3	25
Total Volume	**5**	**6**	**50**
Final Concentration (at 10X)	**Primers: 9 µM; Probe: 2 µM**		

3 Preparing Sample Pre-Mix and Samples

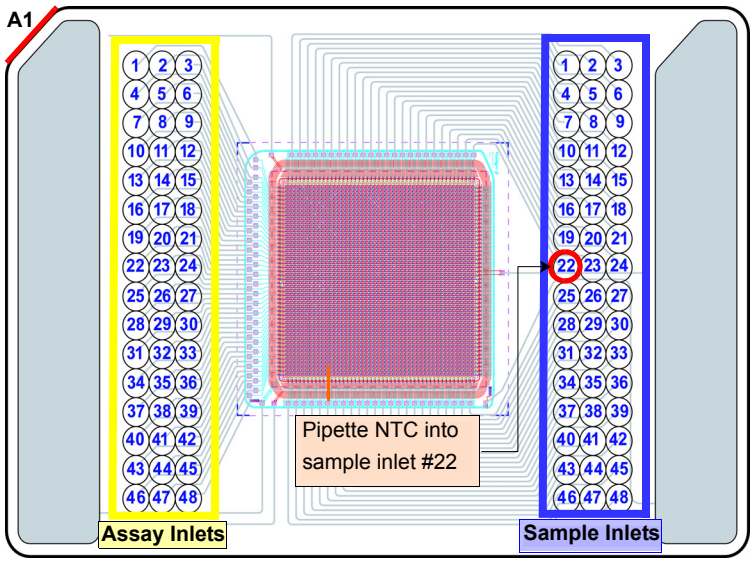
1 Combine components in table below to make Sample Pre-Mix and final Sample Mixture (scale up appropriately for multiple runs).

Component	Volume per Inlet (µL)	Volume per Inlet with Overage (µL)	Sample Pre-Mix for 48.48 (µL) (60 for ease of pipetting)
SAMPLE PRE-MIX			
TaqMan® Universal PCR Master Mix (2X) (Applied Biosystems, PN 4304437)	2.5	3	180
20X GE Sample Loading Reagent (Fluidigm, PN 85000746) ●	0.25	0.3	18
cDNA	2.25	2.7	
Total Volume	**5**	**6**	

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 chip. 3.3 µL of this Sample Pre-Mix can then be aliquoted for each sample.

3 Remove these 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.

Chip Pipetting Map



Technical Support

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4 Loading the Chip

CAUTION! VORTEX THOROUGHLY AND CENTRIFUGE ALL ASSAY AND SAMPLE SOLUTIONS BEFORE PIPETTING INTO THE CHIP INLETS. FAILURE TO DO SO MAY RESULT IN A DECREASE IN DATA QUALITY.

IMPORTANT! FOR UNUSED SAMPLE INLETS, USE 3.3 µL OF SAMPLE PRE-MIX AND 2.7 µL OF DNA-FREE WATER PER INLET. FOR UNUSED ASSAY INLETS, USE 3.0 µL ASSAY LOADING REAGENT AND 3.0 µL OF WATER. DO NOT LEAVE ANY INLETS EMPTY.

IMPORTANT! RUN NTC IN SAMPLE INLET #22.

CAUTION! WHILE PIPETTING, *DO NOT* GO PAST THE FIRST STOP ON THE PIPETTE. DOING SO MAY INTRODUCE AIR BUBBLES INTO INLETS.

1 When the **Prime (113x)** script has finished, remove the primed chip from the IFC Controller MX and pipette 5 µL of each assay and each sample into their respective inlets on the chip.

2 Return the chip to the IFC Controller MX.

3 Using the IFC Controller MX software, run the **Load Mix (113x)** script to load the samples and assays into the chip.

4 When the **Load Mix (113x)** script has finished, remove loaded chip from the IFC Controller MX.

5 Remove any dust particles or debris from the chip surface.

You are now ready for your chip run.

CAUTION! START THE CHIP RUN ON THE BIOMARK INSTRUMENT WITHIN 4 HOURS OF LOADING THE SAMPLES.

5 Using the Data Collection Software

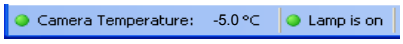
IMPORTANT! BE SURE TO SELECT *ALL* PROBE TYPES PRESENT IN YOUR EXPERIMENT. DATA ARE NOT COLLECTED ON UNSPECIFIED PROBES.

1 Double-click the Data Collection Software icon on the desktop to launch the software.

2 Click **Start a New Run**.

3 Check the status bar to verify that the lamp and the camera are ready.

Make sure both are green before proceeding.



4 Place the loaded chip into the reader.

5 Click **Load**.

6 Verify chip barcode and chip type.
- **a** Choose project settings (if applicable).
- **b** Click **Next**.

7 Chip Run file:
- **a** Select **New** or **Predefined**.
- **b** Browse to a file location for data storage.
- **c** Click **Next**

8 Application, Reference, Probes:
- **a** Select Application— **Gene Expression**.
- **b** Select Passive Reference (ROX).
- **c** Select Assay—**Single Probe**, **Two Probes** or **More than two probes**.
- **d** Select probe types.
- **e** Click **Next**.

9 Click **Browse** to find thermal protocol file— **GE 48x48 Standard v1.pcl**.

CAUTION! MAKE SURE THAT YOU USE A 48.48-SPECIFIC PROTOCOL.

10 Confirm **Auto Exposure** is selected.

11 Click **Next**.

12 Verify the chip run information.

13 Click **Start Run**.

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