Fluidigm® Access Array™ IFC 2-Primer Workflow Quick Reference

PN 68000148, Rev C

For more information see, *Access Array System User Guide, PN 68000158*

1 Priming the Access Array IFC

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

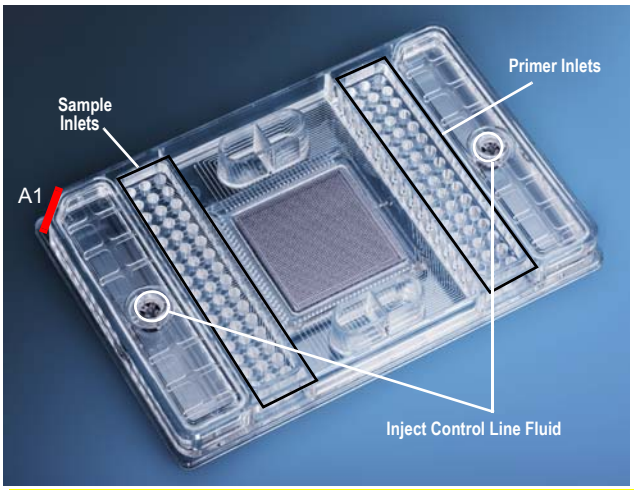
- DUE TO DIFFERENT ACCUMULATOR VOLUMES, USE ONLY 48.48 SYRINGES WITH 300 µL OF CONTROL LINE FLUID (PN: 89000020).
- CONTROL LINE FLUID ON THE CHIP OR IN THE INLETS MAKES THE CHIP UNUSABLE.
- LOAD THE CHIP WITHIN 60 MINUTES OF PRIMING.

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

2 Add 500 µL of 1X Access Array Harvest Reagent (PN 100-1031) into the H1-H4 wells on the chip.

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

4 Place the chip into the ***Pre-PCR*** IFC Controller AX located in the Pre-PCR Lab and run script **Prime (151x)**.



* Please note the location of the sample inlets is different from 48.48 Gene Expression or Genotyping IFCs.

2 Preparing 20X Primer Solutions

Component	Volume (µL)
50 µM TS Forward Primer	8.0
50 µM TS Reverse Primer	8.0
20X Access Array Loading Reagent (PN: 100-0883) ◯	5.0
PCR Certified Water	79
Total Volume	**100.0**

1 Vortex 20X Primer Solutions for 20 seconds and centrifuge for 30 seconds.

NOTE THE FINAL FORWARD PRIMER CONCENTRATION IS 4 µM IN THE 20X PRIMER SOLUTION. THE FINAL REVERSE PRIMER CONCENTRATION IS 4 µM IN THE 20X PRIMER SOLUTION.

NOTE BRING THE 20X ACCESS ARRAY LOADING REAGENT UP TO ROOM TEMPERATURE BEFORE USE.

3 Preparing Samples

1 **Prepare Pre-Sample Master Mix**: In a DNA-free hood, combine the components listed below from the FastStart High Fidelity PCR System, dNTP pack (Roche, 04 738 292 001), with 20X Access Array Loading Reagent and PCR certified water in a 1.5 mL sterile tube (sufficient volume for one chip).

Component	Volume per reaction (µL)	Pre-Sample Master Mix for 48.48 (µL) (60 for ease of pipetting)
10X FastStart High Fidelity Reaction Buffer without $MgCl_2$	0.5	30.0
25 mM $MgCl_2$	0.9	54.0
DMSO	0.25	15.0
10 mM PCR Grade Nucleotide Mix	0.1	6.0
5 U/µL FastStart High Fidelity Enzyme Blend	0.05	3.0
20X Access Array Loading Reagent (PN: 100-0883) ◯	0.25	15.0
PCR Certified Water	1.95	117.0
Total	**4**	**240.0**

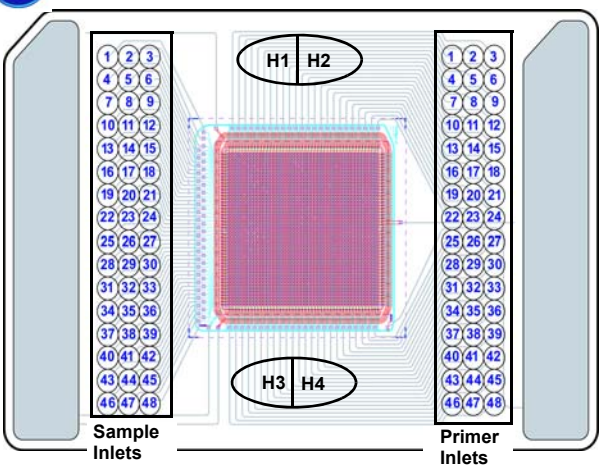
2 Vortex Pre-Sample Master Mix for 20 seconds and centrifuge for 30 seconds before preparing Sample Mix.

3 **Prepare Samples**: For each sample, in an individual microtube or in a 96-well PCR plate, prepare the following solution:

Component	Volume per reaction (µL)
Pre-Sample Master Mix (from above step)	4.0
50 ng/µL Genomic DNA	1.0
Total	**5.0**

4 Vortex Samples for 20 seconds and centrifuge for 30 seconds after all samples are prepared.

Access Array IFC Pipetting Map



4 Loading Samples

CAUTION! PLEASE NOTE CHIP ORIENTATION BEFORE PIPETTING REAGENTS INTO INLETS.

1 Pipette 4 µL of 20X Primer Solution into each of the Primer Inlets.

2 Pipette 4 µL of Sample Mix into each of the Sample Inlets.

3 Place the chip into the ***Pre-PCR*** IFC Controller AX in the Pre-PCR Lab and run script **Load Mix(151x)**.

5 Thermal Cycling the IFC

1 Place the chip onto one of the following and run PCR by selecting protocol specified below.

- For the **Fluidigm FC1™ Cycler**, choose the **AA 48X48 Standard v1** protocol. Refer to the *Fluidigm FC1 Cycler Usage Quick Reference*, PN 100-1250, for more information.
- For the **Fluidigm Stand-Alone Thermal Cycler** , choose the **AA48v1** protocol. Refer to the *Fluidigm Stand-Alone Thermal Cycler Usage Quick Reference*, PN 68000111, for more information.
- **BioMark™ System** (Please contact Fluidigm Technical Support)

6 Harvesting the IFC

1 After PCR has finished, move Access Array chip into the Post-PCR Lab for harvesting.

2 Remove remaining 1X Access Array Harvest Reagent from H1-H4 wells.

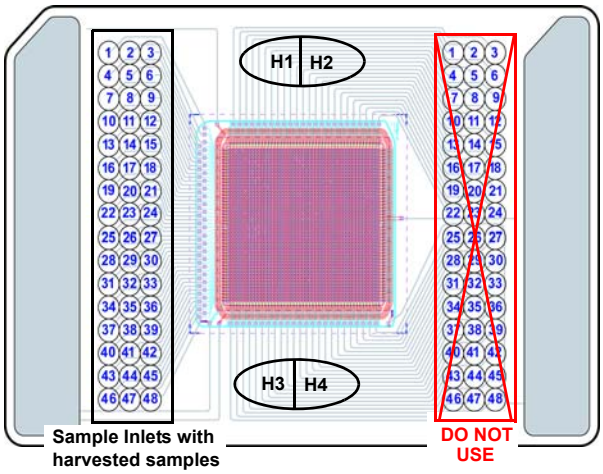
3 Pipette 600 µL of 1X Access Array Harvest Reagent into the H1-H4 wells.

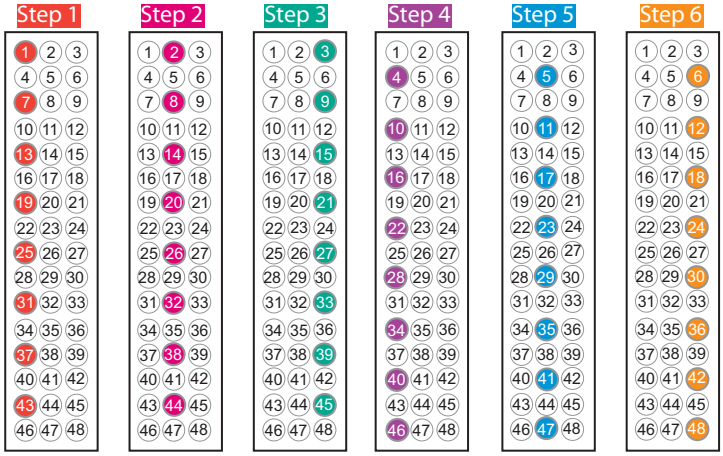
4 Pipette 2 µL of 1X Access Array Harvest Reagent into each of the Sample Inlets on the chip.

5 Place the chip into the ***Post-PCR*** IFC Controller AX located in the Post-PCR Lab and run script **Harvest (151x)**.

6 When the **Harvest (151x)** script has finished, remove the IFC from the ***Post-PCR*** IFC Controller.

7 Label a 96-well plate using the barcode number on the Access Array chip. Carefully transfer the harvested samples into columns 1-6 of the pre-labelled 96-well PCR plate. Follow the same pipetting pattern you used to transfer samples from the 96-well plate to the IFC.





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