



MI PathArray™ Static Hybridization Protocol

Components for this protocol (available from MI)

ITEM	CATALOG #
MI PathArray slides	Various options
HybriWell Static 4-well Gaskets	MISMG004
Gasket Sealing Tool	MIGST001
Hybridization kit (optional)	MIHK001

Other materials

Prepared, labeled cDNA
Spectrophotometer
1.5ml microcentrifuge tubes (use amber tubes, if possible)
Dry Bath or Heat Block, set to 98°C
Hybridization Oven, set to 42°
Centrifuge

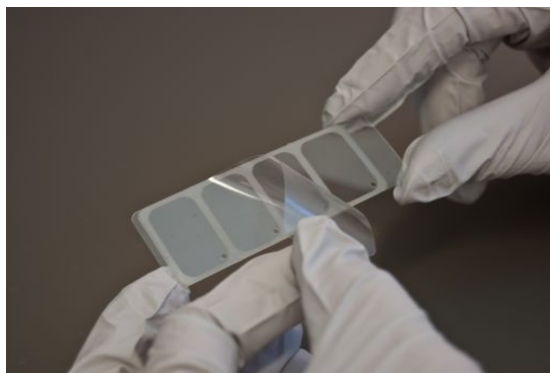
Rotating platform
Containers for mixing hybridization wash buffers
Slide washing vessels/Coplin jars
Microcentrifuge

A. Application of the HybriWell™ gasket to the MI PathArray slide

***Note:** *If you have ordered MI PathArray slides with applied gaskets you may skip to the section titled "Prepare Sample Solution."*

1) Identify the printed surface of the MI PathArray slide. The microarray is printed on the surface of the slide where the barcode numbering is legible (barcode face up).

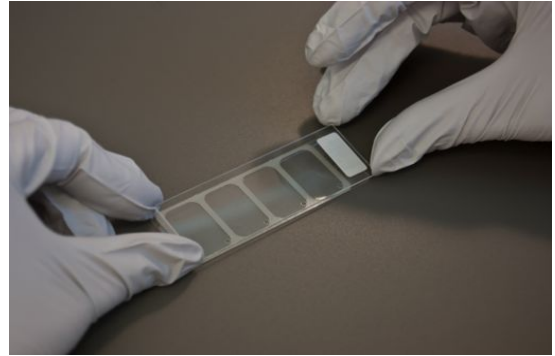
2) To apply the 4-chamber adhesive gasket, first peel off the clear release liner from the gasket.



3) Place the gasket on a flat, dry surface with the adhesive side up and longer tab end to the right.

***Note:** The longer tabbed end of the HybriWell gasket pairs with the barcode area on the slide. Be certain to avoid placement of the adhesive walls of the gasket over the barcode. To do so may compromise the seal.

4) Invert the microarray slide so that the printed face is toward the HybriWell gasket (face down). Keep the barcode end of the slide to the right to pair with the longer tabbed end of the gasket.



5) Carefully match the edges of the gasket and the microarray slide so they align evenly without overhang.

6) Seal the adhesive edges of the gasket by tracing the well outlines using a flat-edged sealing tool. Take care to remove any air bubbles and to avoid printed array areas.



7) Visually inspect the seal quality prior to loading sample solution into the wells.

8) Use the sealing tool to seal any edges that appear loose.

B. Prepare Sample Solution

9) Dissolve 25-50ng pre-labeled cDNA test sample in 170 μ l of MI Hybridization Buffer in a 1.5ml amber microcentrifuge tube. Repeat for all test samples.

10) Mix gently by tapping the tube and microfuge tube briefly to collect contents.

11) Heat each Sample Solution in a dry bath or heat block at 98°C for 3 minutes.

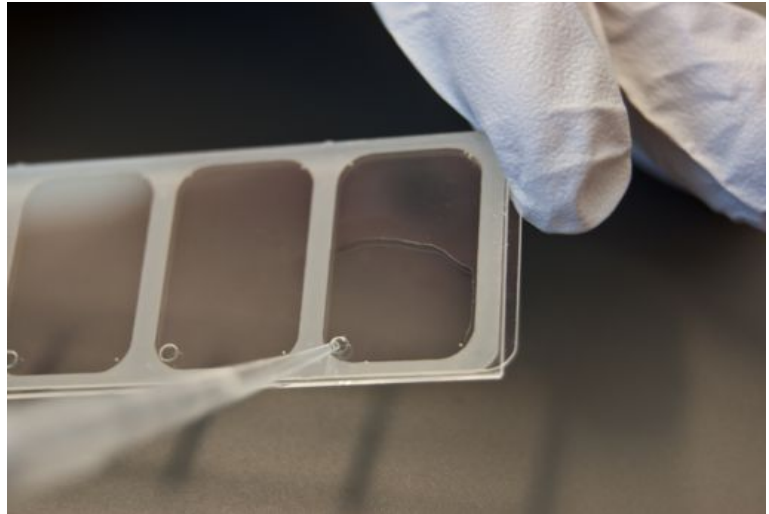
12) Microfuge tube briefly.

C. Apply the Sample Solution for Static Hybridization

***Note:** The capacity of each chamber of the static HybriWell gasket is 42 μ l.

13) Hold the prepared MI PathArray at an angle so the large fill port is lower than the 3 smaller vent ports.

14) Slowly pipette 42 μ l Sample Solution through large port of the HybriWell chamber. Do not allow air bubbles to form. Continue to fill the chamber with the entire 42 μ l Sample Solution even after the chamber appears to be full.



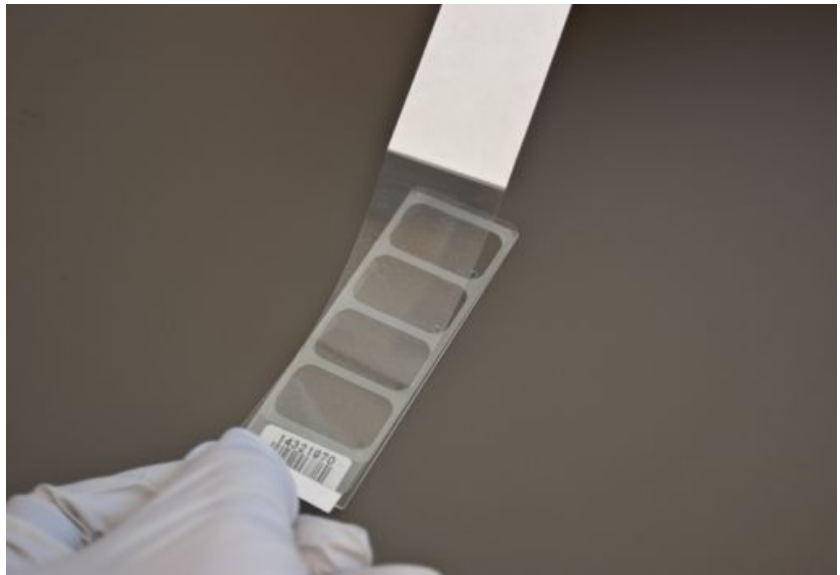
Slide is tilted while adding Sample Solution into the hyb chamber

***Note:** If large bubbles form while filling the gasket, you may apply very light pressure to the clear window of the gasket to dislodge the bubbles.

15) After all wells are filled with their respective Sample Solutions, place the array on a flat surface.

16) Carefully remove the paper backing of the gasket sealing strip and align the seal to cover the entire surface of the gasket.

17) Apply the seal and ensure the fill port and small vent ports of all wells are sealed.



***Note:** if a wrinkle or misalignment is made, DO NOT attempt to peel off the seal strip as this may cause a disruption of the gasket or evacuation of liquid from the chambers. Instead, place a second seal strip over the gasket area to block ports and vents for hybridization. If a second seal strip is not available, a small drop of mineral or a piece of translucent tape (office tape) can be placed over any open port.

D. Incubation of the MI PathArray slide

18) Incubate the MI PathArray slide for approximately 16-24 hours with the printed array face-up on a flat surface inside a humidified hybridization chamber or incubator set to 42°C. Protect from light.

19) Do not allow the array to cool or the surface to dry prior to washing.

E. Post Hybridization Washing of MI PathArray

Note: Prepare all wash solutions prior to removing the array from the hybridization chamber. You will need a minimum of 50ml per array for each wash step but this is highly dependent upon the wash chamber you choose.

Wash Solution 1: 447.5ml deionized water,
50ml Wash Reagent A,
2.5ml Wash Reagent B;
Warm Wash Solution 1 to 42°C

Wash Solution 2: 1425ml deionized water,
75ml Wash Reagent A

Wash Solution 3: 300ml Wash Solution 2,
1200ml of deionized water

20) Preheat Wash Solution #1 to 42°C.

21) Fill a slide-washing vessel, such as a Coplin jar, with warmed Wash Solution #1 with enough volume to cover the entire array surface.

22) Remove the Hybriwell gasket by grasping the tab end of the Hybriwell firmly and slowly peeling it away from the slide. Take care not to touch the hybridized array surface. Discard the HybriWell.



23) Immediately immerse the array in warmed Wash Solution #1.

24) Incubate the MI PathArray slide at 42°C with gentle agitation on a rotating platform for 5 minutes.

25) Transfer the MI PathArray slide to Wash Solution #2.

26) Incubate MI PathArray slide for 4 minutes with gentle agitation at RT.

27) Transfer the MI PathArray slide to Wash Solution #3.

28) Incubate the MI PathArray slide in Wash Solution #3 for 4 minutes with gentle agitation at room temperature.

F. Drying the Array Surface

***NOTE:** *Do not allow the array to air dry as this may cause spotting and excess background.*

29) Remove the MI PathArray slide from Wash Solution #3 and immediately dry the array surface under a stream of clean nitrogen or by centrifugation at 200 x g for 5 minutes.

30) Take measures to avoid introducing dust or abrading the surface of the slide in handling.

Processed arrays should be scanned immediately or stored in a lightproof slide holder until ready to scan. For best results, arrays should be scanned within 2 hours of processing.

References

“How to Use HybriWell™” Product Insert Instruction Manual, GRACE Bio-Labs, Inc.