Microarrays Inc

MI Ready Array Instruction Manual
Revision 1.03 08/08

Array Storage

When properly stored, arrays should remain hybridization competent for at least six months. Store arrays

- in their original packaging (including the metallic outer pouch)
- in dry or desiccated atmosphere
- at room temperature
- protected from light.

Microarrays Inc ships arrays in desiccated, re-sealable storage pouches that are ideal for this purpose. Both the storage pouches and slide storage boxes they contain are manufactured from materials that minimize out-gassing and block exposure to contaminants that can elevate background levels. When accessing arrays, remove only the arrays you plan to immediately use and return the remaining arrays to the pouch quickly. Leave the desiccant pack in place.

Prehybridization

Prehybridizing the arrays will lower background levels and improve sensitivity and signal to noise ratios by reducing target DNA affinity to non-probe areas of the array. Prehybridization must be performed immediately prior to hybridization.

1) Prepare an appropriate volume of Prehybridization Solution
    a. 5xSSC, 0.1% SDS, and 0.1% BSA.
        Any high quality reagents will work, some suitable choices include: sodium dodectyl sulfate, Sigma part#L4509, and bovine serum albumin, Sigma part#B4287.
    b. The volume of prehybridization solution required is normally a function of the container and rack. The level of prehybridization solution must extend to the barcode label. Use at least 10ml of solution per slide.
    c. The choice of hybridization container is not critical but a suitable container capable of preparing up to 10 slides in a 100ml volume can be purchased through VWR (Part#25460-907 TPX staining jar).
2) Preheat the solution to 55 °C.
3) Immerse arrays in the prehybridization solution.and gently rock for 30 min.
4) Rinse the arrays with 5 exchanges of dH_20.

a. NOTE: It is critical that the warm prehybridization solution not be allowed to dry on the array (as it is prone to do). One way to minimize this risk during the initial rinse is to place the slide container in a sink and flood with dH2O.
b. Each rinse step should be accompanied by at least 60 seconds of gentle rocking.
c. All the prehybridization solution must be removed. Over washing is preferred, as it will not harm the arrays.
d. NOTE: Do not allow the arrays to air dry! Dry the arrays by centrifugation or by blowing with ultra-pure compressed gas.
e. There should be no visible material remaining on the array surface after drying. Visible material will usually result in high background levels.

Standard Hybridization

The array substrate and probe attachment chemistry are extremely stable and compatible with most hybridization conditions including high stringency conditions, high formamide concentrations, and/or elevated temperatures.

This standard hybridization protocol is included that should be suitable for many applications.

1) Prepare a 2x concentration of hybridization solution
    a. 40-70% deionized formamide, 10xSSC, 0.2% SDS, and (optional) 0.02% sheared salmon sperm DNA.
    b. Any high quality reagents will work, some suitable choices include: deionized formamide, Sigma part# F9037, sodium dodectyl sulfate, Sigma part#L4509, and salmon sperm DNA, Sigma part#D1626, water, Sigma part#W4502.
    c. The volume of hybridization solution required depends on the type of hybridization (static or mixing apparatus) and type of coverslip.
        i. A standard flat 24mm x 60mm coverslip (Corning part#2935-246) requires approximately 50 μl.
        ii. An elevated 25mm x 60mm cover slip (Erie Lifterslip part#2-4789) requires 85 μl.
        iii. Automated hybridization apparatuses will typically require even greater volumes.
2) Combine labeled target solution and 2x hybridization solution.
3) Adjust final volume to achieve 1x concentration with nuclease free dH_2O.
4) Mix reagents in tube
5) Denature hybridization solution target at 95°C for two minutes.
6) If performing a coverslip hybridization, rinse the coverslip in 70% ethanol prior to use and allow to dry.
7) Pipette hybridization mixture onto the middle of the array and slowly lower the cover slip to avoid the inclusion of air bubbles. Very small air bubbles will usually work themselves out and should not cause a problem.
8) Immediately place the array-coverslip combination in a suitable hybridization chamber prepared with suitable volumes of blank hybridization buffer to prevent evaporation.
    a. Evaporation of hybridization solution from the cover slip will typically cause irreversible binding of labeled targets to the array and elevate background levels and reduce target available for probe hybridization. Any good hybridization chamber that prevents evaporation will work; one suitable choice is Corning part #2551.

9) Place the sealed hybridization chamber in a gently shaking water bath or incubation oven at 42 °C for 12 to 16 hours.

Additional notes on hybridization - The quantity of labeled target DNA required to achieve a good hybridization signal is influenced by a number of factors including the type, source, and quality of RNA and the type of fluorescent labeling method employed. If you do not have a well-established protocol that achieves the required quantities of labeled target, the quantity of target and label incorporation can be determined through absorption spectrophotometry. Here are some general guidelines for conventional Cy-dye labeled targets.

i. For targets derived from total RNA, try to achieve 60 pico-moles of each (Cy-3 and Cy5) dye incorporated into the labeled target mixture.
ii. For targets derived from selected mRNA, try to achieve 15 pico-moles of each (Cy-3 and Cy5) dye incorporated into the quantity of labeled target mixture.
iii. The quantities of labeled target DNA can be determined by the Beer-Lambert equation.

$$[\text{target ssDNA (ug/ml)}] = (A_{260})*(\epsilon_{ssDNA})*(L)$$

Where A_{260} equals the absorbance at 260_{nm}, ϵ_{ssDNA} equals 37 (μg/ml 1/cm) the extinction coefficient of single stranded cDNA targets, and L equals the path length in cm.

iv. The number of pico-moles of incorporated Cy-3 and Cy-5 dye can be determined by the same method

$$\text{picomolar Cy-dye concentration} = (A_{Cy\text{-}dye})*(\epsilon_{Cy\text{-}Dye})*(L)$$

$$\#\ \text{pmol} = (\text{picomolar Cy-dye Molar Concentration}) * (\text{Volume of solution})$$

Where $A_{Cy\text{-}Dye}$ equals the absorbance at 550_{nm} and 649_{mn} for Cy-3 and Cy-5 dyes respectively, $\epsilon_{Cy\text{-}Dye}$ equals 0.15 and 0.25 (Molar 1/cm) the extinction coefficient of Cy-3 and Cy-5 respectively, and L equals the path length in cm.

Post Hybridization Washing

Post hybridization washing is required to remove un-hybridized target DNA from the array. It is <u>critical</u> that the hybridization solution not be allowed to dry on the arrays as they are removed from the hybridization chamber and transferred to the washing containers. Multiple containers are preferred to provide thorough exchange of wash buffers at each successive washing steps.

NOTE: The volume of wash solutions required depends on the choice of slide rack and container. Try to achieve at least 10ml of wash buffer per slide.

1) Prepare one volume of wash solution #1:
    a. 2xSSC, 0.1% SDS at 42 °C,
2) Prepare one volume of wash solution #2:
    a. 0.1xSSC, 0.1% SDS at room temperature
3) Prepare two volumes of wash solution #3:

a. 0.1xSSC at room temperature.

4) Remove the array from the hybridization chamber and rapidly immerse in wash solution #1, leaving the cover slip in place.
5) Mix with gentle agitation 5 minutes.
    a. If the cover slip has not fallen off after 30 seconds of washing, gently withdraw and reinsert the array into the wash solution or use a pair of forceps to facilitate its removal.
6) Transfer the array to wash solution #2
7) Mix with gentle agitation 5 minutes.
8) Transfer the array to the first volume of wash solution #3
9) Mix with gentle agitation 1 minute.
10) Transfer the array to the second volume of wash solution #3
11) Mix with gentle agitation 1 minute.
12) NOTE: Do not allow the arrays to air dry! Dry arrays quickly by centrifugation or by blowing dry with ultra-pure compressed gas.

Post Hybridization Analysis - NOTES

- Cy-dyes are susceptible oxidative degradation and bleaching and are best scanned immediately following the post hybridization washing.
- If arrays must be stored before scanning place them in a desiccated chamber protected from light.
- Scan the arrays and adjust the scanner's Cy-3 and Cy-5 sensitivity to achieve the best possible signal to noise ratios without signal saturation.
- Be sure to cross reference your results with the quality control report associated with your specific array lot. The report contains information about all the probe depositions in your specific lot of arrays and their respective levels of hybridization-competent DNA assayed by Microarrays Inc's proprietary Veriprobe™ assay.