

Notice to the User



It is important that users read the entire manual before commencing work.

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User Guide and Technical Support

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Feedback

We welcome your feedback regarding our products and this manual.

Please contact us at:

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All comments are welcome.

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Last updated December 2008

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Thank You

Phalanx Biotech Group would like to extend special thanks to our customers who have provided feedback that enabled us to improve the miRNA OneArray User Guide.

miRNA OneArray™

User Guide

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Getting Started

Please read the introductory information below to help familiarize yourself with OneArray™ before use.

Product Contents

- miRNA OneArray™ DNA Microarrays
- OneArray™ 1.5X Hybridization Buffer I
- miRNA OneArray™ Hybridization Buffer II
- Spare round cap tube
- miRNA OneArray™ User Guide
- Spotted Region Guide
- Product Support CD, which contains the following:
 - Sample Images
 - miRNA OneArray™ gal file(s)
 - miRNA OneArray™ probe sequences
 - miRNA OneArray™ annotation file
 - miRNA OneArray™ microarray layout
 - miRNA OneArray™ Control Probe list
 - miRNA OneArray™ User Guide (electronic version)

Other Necessary Apparatus (Not Supplied)

Apparatus

- Water bath/heating block
- Powder-free gloves
- Clean, blunt forceps
- Micropipettors
- Sterilized and nuclease-free pipet tips
- Sterilized and nuclease-free microcentrifuge tubes
- High-speed microcentrifuge
- Low-speed tabletop microcentrifuge with slide holder attachment
- Vortex mixer
- Hybridization oven
- Hybridization accessories: chamber cover slides, etc.
- Rectangular slide staining dish and slide rack for washing microarrays
- Thermocycler
- Microarray scanner for standard 1” x 3” format (see Table 8 under “miRNA OneArray™ Microarray Scanner Specifications” for a list of compatible scanners)
- Hybridization systems (optional)
- Automated hybridization station (optional)

Other Necessary Reagents (Not Supplied)

Reagents

- De-ionized nuclease-free water
- Cyanine 3- or 5-labeled miRNA sample
- 20X SSPE stock solution, sterile filtered:
 - 3 M Sodium chloride
 - 3 M Sodium citrate
- Wash Solutions, sterile filtered (four types, approximately 250 mL of each is required per experiment):
 - Wash I: 2X SSC, 0.2% SDS
 - Wash II: 2X SSC
 - Wash III: 0.2X SSC
 - **NOTE:** SDS must be molecular biology grade.
- 100% Ethanol
- Pre-hybridization Buffer, prepared and sterile filtered immediately prior to pre-hybridization:
 - 5X SSPE, 0.1% SDS, 1% BSA
 - **NOTE:** BSA must be molecular biology grade.
- Deionized **formamide** to be added to the OneArray™ 1.5X Hybridization Buffer prior to use (see Step 4A).

Important Notes on Microarray Handling and Storage

Storage Conditions

- Store unopened miRNA OneArray™ product at room temperature.
- Store opened miRNA OneArray™ product at 4°C.
- Store OneArray™ 1.5X Hybridization Buffer I at room temperature.
- Store miRNA OneArray™ Hybridization Buffer II at -20°C temperature.

NOTE: If the product is received with an open bag, please contact Phalanx Biotech Customer Service for an immediate replacement.

Handling Microarrays



Please read this section carefully and follow the instructions!

- Polynucleotide probes are printed on the side of the slide with the barcode.
- To avoid irreparable damage of the printing area, **do not touch** the surface with bare hands, or with any other objects.
- Whenever possible, handle microarrays with clean blunt forceps to avoid contamination.



Open arrays should be used within a week.

Product Description and Overview

miRNA OneArray™ microarrays are made of polydeoxynucleotide probes spotted onto a proprietary chemical layer coated on top of a 1" x 3" (25 mm x 75 mm) standard-format microarray glass slide.

Each probe is spotted onto the array in a highly consistent manner using a proprietary, non-contact spotting technology adapted for microarray manufacturing.

Using miRNA OneArray

This section provides you with detailed information about how to perform the steps necessary to complete the hybridization process to study gene expressions using the miRNA OneArray™ microarray.



Follow these detailed steps *exactly* to achieve the best experimentation results.

- **Step 1:** [Prepare the RNA Sample](#)
- **Step 2:** [Label the Target](#)
- **Step 3:** [Pre-Hybridize the Microarray](#)
- **Step 4:** [Perform the Hybridization Protocol](#)
- **Step 5:** [Wash the Hybridized Microarray](#)
- **Step 6:** [Scan and Extract Gene Expression Results](#)
- **Step 7:** [Check Control Probe Data](#)

Step 1:**Prepare the RNA Sample**

High-quality, intact RNA is essential for all gene expression microarray experiments.

There are many different RNA isolation protocols and commercially available RNA isolation kits. You should choose a solution that meets your specific needs. Kreatech, Stratagene, Ambion, Invitrogen and other reagent companies offer many different RNA isolation products. For more information, you can visit each company's Web site.

Once the RNA samples are isolated, you must confirm the quantity and quality of the samples. Similarly, many different protocols are available and you should choose a solution that is suitable for your needs.

For faster and more automated RNA analysis, you may want to consider the "No Cuvettes" Spectrophotometer from NanoDrop[®], or the 2100 Bioanalyzer from Agilent Technologies. For more information, visit each company's Web site.

Step 2: Label the Target

General Guidelines for Target Labeling

There are many commercially available labeling kits for microarray analysis. Select a labeling kit or labeling method that is most suitable for your specific needs.

You may want to confirm the quality of the labeled target with the “No Cuvettes” Spectrophotometer from NanoDrop®.

Follow the instructions provided by the reagent supplier. Indirect labeling with NHS ester dye is recommended. Table 3, below, contains a list of products that have been tested for use with miRNA OneArray™.

Manufacturer	Product Name and Description
Ambion®	miRVana™ miRNA Isolation Kit
Ambion®	miRVana™ miRNA Labeling Kit

For RNA labeling, 1-5 µg enriched miRNA is recommended. Smaller volumes can lead to significant loss of sample and may increase the concentration of contaminants in the labeled RNA sample, leading to higher background signal.

It is best to use RNA as soon as possible after labeling, as exposure to air and light can reduce the signal of some dyes. If it must be left overnight, it is best to aliquot your labeled RNA and store in the dark at -80°C. Avoid thawing and re-freezing RNA if possible, as freeze-thaw cycles can damage the RNA.

Step 3:

Pre-Hybridize the Microarray

General Instructions



miRNA OneArray™ requires a pre-hybridization step prior to hybridization of the labeled target. The pre-hybridization step reduces background signals and increases the performance of the microarray. Complete the pre-hybridization step by carefully following the instructions below.

- 1) Warm the pre-hybridization solution (5X SSPE, 0.1% SDS, and 1% BSA) to 42°C.
- 2) Pour 25 ml room temperature 100% ethanol into the spare array tube.
- 3) Preheat the miRNA OneArray™ (s) in the round cap tube at 60°C for 10 min (hybridization oven recommended).
- 4) Remove the miRNA OneArray™ (s) from the round cap tube, place in the two outermost slots inside the tube containing 100% ethanol, close the cap, and let sit for approximately 15 sec.
- 5) Shake the round cap tube for 20 sec.
- 6) Remove and thoroughly rinse each array with deionized water to remove any residual ethanol.
- 7) Carefully and slowly, fully submerge the miRNA OneArray™ in an abundant amount of pre-hybridization solution for 2 hr at 42°C (35 ml is sufficient if using a round cap tube).



Try to insert the slides into the correct position the first time. Avoid inserting and removing the slides more than once in the pre-hybridization buffer.

- 8) After 2 hr, transfer the slide(s) to room temperature, distilled water and wash gently for 2 min.
- 9) Spin dry the slide(s) for 2 min. Store in a dry, dark place until hybridization. It is recommended that you use the slides in the hybridization protocol within 1 hr of completing the pre-hybridization process.

Step 4:

Complete the Hybridization Protocol

Once you have completed the pre-hybridization step using one of the methods outlined in the [Step 3: Pre-Hybridize the Microarray](#) section, you are ready to complete the hybridization protocol.

There are many different hybridization protocols, apparatus, and instruments available that may be compatible for use with the miRNA OneArray™ microarray. Detailed instructions for using the glass cover slide method are described below.

For best performance and consistent hybridization results, it is recommended that you use the OneArray™ Hybridization Buffers included with this product to complete the hybridization process.

Step 4A: → Prepare Hybridization Solution Using the OneArray™ 1.5X Hybridization Buffer I (Included)

IMPORTANT!



For correct use of this buffer, you must add a specific amount of formamide and labeled target. Please follow the instructions below carefully.

- 1) Spin down the stock OneArray™ 1.5X Hybridization Buffer I (~410 µl in each tube).
- 2) Add 90 µl of deionized formamide.

3) Warm the mixture to 42°C to completely dissolve the solution. Mix thoroughly.

Yield: 500 µl of OneArray™ 1.5X Hybridization Buffer I.

Step 4B:→ Complete the Hybridization Using the Glass Cover Slide Method

Hybridization Using Labeled Targets from Enriched miRNA Labeling Approaches

NOTE: The protocol that follows is for use with a single array. Take care when using a coverslip that the solutions from neighboring arrays printed on the same slide do not mix.

NOTE: If you perform hybridization using methods other than the basic glass cover slide method, it is recommended that you validate the protocol experimentally.

To complete this step, you will need to select a type of chamber or glass cover slide. Table 5, below, contains a list of products that have been tested and confirmed compatible for use with the OneArray™ Buffer.

Manufacturer	Product Name
Corning®	Cover Glass (22 X 22 mm)
Erie Scientific Company®	mSeries LifterSlip™ 22X25I-M5226
BioMicro™	MAUI Mixer DC**
Phalanx Biotech Group	OneArray Dual Hyb Chamber

**The MAUI DC Chamber requires the use of the MAUI Hybridization System. Details can be found at the manufacturer's website: <http://www.biomicro.com>.

- 1) Ensure your work and experimentation area, as well as the miRNA OneArray™, are clean before adding the Hybridization Buffers solution to the target array.
- 2) Pre-warm the OneArray™ 1.5X Hybridization Buffer I with formamide at 37°C for 10 minutes.
- 3) Mix your labeled miRNA sample with nuclease-free H₂O to yield a final volume of 17 µL.

NOTE: For enriched miRNA, it is essential to use at least 1 µg of labeled miRNA. If you are performing a dual-dye experiment, use at least 1 µg of *each* labeled miRNA sample.

- 4) Prepare the hybridization mix in a 0.2 ml Eppendorf tube according to the Table 6, below.

Table 6: Hybridization Mix Measurements	
For each array: 55 µl	
Component	Final Volume
1.5X miRNA OneArray™ Hybridization Buffer I	37 µl
miRNA OneArray™ Hybridization Buffer II	1 µl
Target preparation plus nuclease-free ddH₂O	17 µl

- 5) Heat the mixture to 95°C for 2 minutes (thermocycler recommended).
- 6) Maintain the mixture at a temperature of 60°C until pipetting onto the array (thermocycler recommended¹).

¹ It may be helpful to set a Denature program in the thermocycler as follows:
 95°C – 2 minutes
 60°C – Hold

- 7) Place the miRNA OneArray™ slide, bar code up, atop the “Probe Printed Region Guide” (included, see Figure 1).

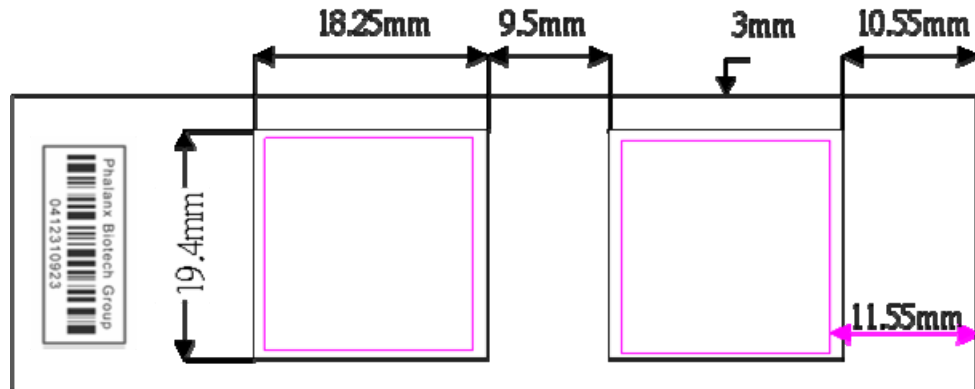


Figure 1: miRNA OneArray™ Microarray Glass Slide with “Probe Printed Region Guide” Plastic Underlay

- 8) Pipette the hybridization mixture onto the spotted region of miRNA OneArray™ Microarray. Avoid creating any bubbles.
- 9) Carefully place the glass cover slide over the spotted area in an even manner.
- 10) Place the entire labeled target plus the microarray set-up into a closable, chambered box* that is humidified by 2X SSC buffer in the 37°C oven for 14 to 16 hours. A sealed chamber ensures that the appropriate humidity level is maintained during incubation. (See Figure 2).

Figure 2, below, provides an illustration of Step 4C, where the hybridization protocol is completed using the glass cover slide method, and specifically, the miRNA OneArray™ Microarray is placed into the chambered box.

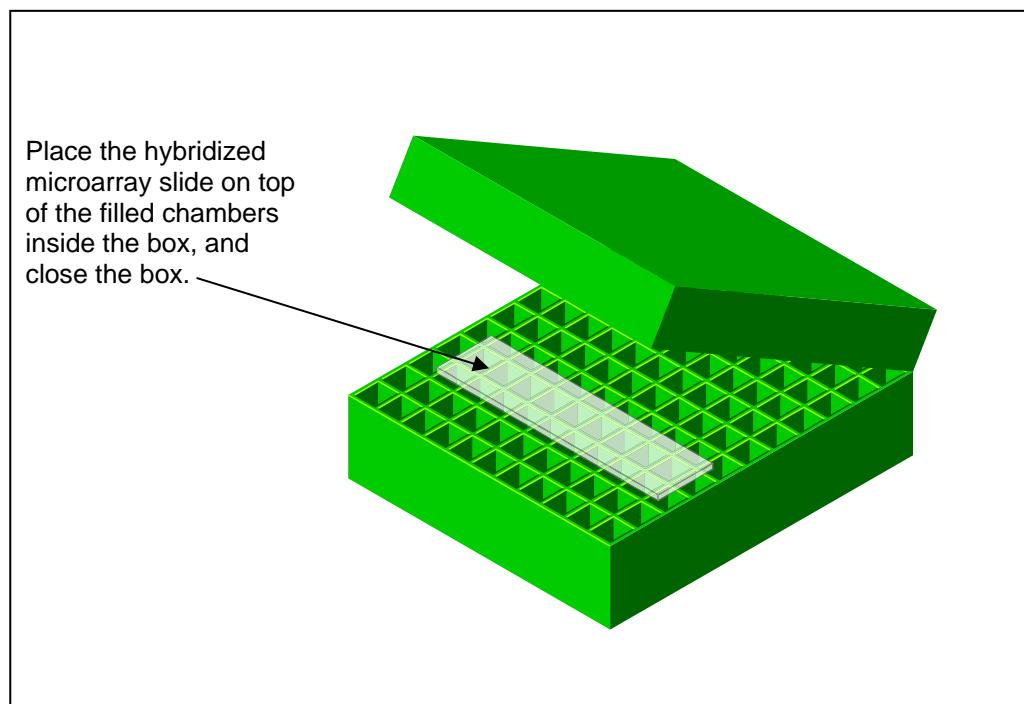


Figure 2: Step 4B → Glass Slide Inside Chamber Box²

² The Hinged 100-Place Storage & Freezer Polypropylene Box from USA Scientific has been used to complete this step with frequent success. The small (approximately ½ inch x ½ inch) chambers within the box are filled about ¾ full of buffer, then the microarrays are laid on top of the chambers. The box is then closed and placed inside the oven. For information about this product or other USA Scientific products, access their Web site at: www.usascientific.com

Step 5:

Wash the Hybridized Microarray

IMPORTANT!



Washed and dried microarrays should be scanned within a couple of hours.

NOTE: Do not allow the microarray(s) to be exposed to air for a significant amount of time; otherwise, an increased fluorescent background signal could appear.

- 1) Submerge the entire labeled target and microarray set-up with the cover slide still intact into a large container filled with 37°C 2X SSC, 0.2% SDS solution.
- 2) Carefully remove the cover slide from the glass by gently shaking the glass slide so that the cover slide is freed while the slide is submerged.

NOTE: At this stage, the microarray has the highest concentration of unhybridized target and dye. Transfer the array quickly to the slide rack to minimize exposure to air.

- 3) Wash the slide(s) in the “rectangular, slide staining dish and slide rack” with the excess amount of pre-warmed 2X SSC, 0.2% SDS solution for 5 min at 37°C.
- 4) Transfer the slide rack to a second slide staining dish that contains 2X SSC solution and wash for 5 min at room temperature.
- 5) Transfer the slide rack to a third slide staining dish that contains 2X SSC and wash for 5 min at room temperature.
- 6) Rinse each array carefully with 0.2X SSC using a squeeze bottle.
- 7) Spin dry with a centrifuge for at least one minute.
- 8) Keep the microarray dry and in the dark until ready to scan.

Step 6:**Scan and Extract Gene Expression Results**

There are many scanners available to extract signals from miRNA OneArray™. Data extraction using GenePix™ 4100 from Molecular Devices is described below. Please refer to the respective company product instructions for appropriate use.

For a list of scanners that are compatible with the miRNA OneArray™, please refer to Table 8, below.

NOTE: The performance of each scanner may differ. Therefore, to ensure best results, it is recommended that the scanner be adjusted based on standard microarray calibration parameters. Turn on and warm up the scanner for the duration according to manufacture instructions for the scanner.

Use the .gal file and Gene List provided with this product, or refer to our Web site at:

www.phalanxbiotech.com

miRNA OneArray™ Microarray Scanner Specifications

Select and use a microarray scanner that meets the specifications below.

Microarray Scanner Specifications

Format capabilities: 1" x 3" (one inch by three inch) glass slide

Molecular capabilities: Able to accurately detect, activate and read Cy3 and Cy5 fluorescent molecules

Table 8, below, contains a partial list of microarray scanner products that are compatible for use with the miRNA OneArray™ microarray. Please refer to the respective company website for more information about the products listed below.

Table 8: Compatible Microarray Scanners

Manufacturer	Product Name and Description
Molecular Devices	Axon GenePix® 4000, 4100, and 4200 series
Genomic Solutions,® Inc.	GeneTAC™ 2000
Perkin Elmer,® Inc.	ScanArray™ 5000
TECAN®	LS 200/300/400
Agilent Technology	DNA Microarray Scanner G2565B

Step 7:**Check the Control Probe Data**

miRNA OneArray™ Microarrays contains built-in control probes for performance monitoring of the hybridization process. They are used to confirm or deny whether the experiment was completed successfully. Please visit

<http://www.phalanxbiotech.com/Support/Support.html>

for more detailed information about the experimental controls on your miRNA OneArray™ product.

Additional information about the control probes is included on the Product Support CD, and on our Web site at:

www.phalanxbiotech.com

