GenoSensor Corporation

GenoExplorer™ miRNA Full Kit

Catalog #'s 1101C – 1199C

Version C
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User Manual

Table of Contents

Literature Citation
When describing a procedure for publication using these products, we would appreciate that you refer to them as the GenoExplorer™ miRNA Full Kit that includes the Labeling Kit and the Biochips Kit.

Patents and Trademarks

Product Overview

The GenoExplorer™ miRNA full kit contains the chips, the labeling system, the reagents for hybridization assay and wash, and the .GAL file for gene positioning on the array and data analysis.

The GenoExplorer™ miRNA Labeling Kit

- **Selective and Uniform Labeling**

GenoExplorer™'s labeling system employs a 5'end ligation approach. This offers greater selectivity in the labeling of RNA compared with more traditional 3' approaches since RNA molecules with capped 5' ends (*e.g.*, intact mRNA) will not be labeled. The ligation reaction also ensures that the labeling is uniform in that a single adaptor is attached to each RNA molecule with an uncapped 5'end. GenoExplorer™ currently offers a biotin label.

- **Simplified Sample Prep Protocol**

With GenoExplorer™'s more selective labeling system, RNA samples do not require the additional miRNA/small RNA isolation step as some labeling protocols recommend. Users can begin their labeling reaction with total RNA. It is important, however, to ensure that small RNA is retained during the initial RNA extraction procedure.

GenoExplorer™ Biochips

- **Comprehensive Probe Content**

GenoExplorer™ chips consist of probes for all miRNAs as registered and annotated in the miRBase at The Wellcome Trust Sanger Institute. A key differentiator of the GenoExplorer™ chip's content is the inclusion of probes for precursor miRNA as well as mature miRNA. The content also includes probes for positive controls (*e.g.*, 5S rRNA, tRNAs, U6) as well as negative controls.

- **Species-Specific and Customizable Chip Formats**

GenoExplorer™ chip products come in several formats. Check our website for updates on the species-specific chips available. Orders of other species are also available upon request.

- **Probe Redundancy and Normalization**

Each probe is printed in triplicate on an array for computing averages. Positive control probes are used for normalization and assay quality control.

- **3-D Gel Matrix for Optimal Hybridization Environment**

The surface of the GenoExplorer™ chip is composed of a 3-D gel matrix, which provides a more optimal hybridization environment by extending the probe into the solution and increasing its access to the target.

GenoExplorer™ Reagents for Hybridization Assay

- **Optimized Hybridization and Wash Buffers**

GenoExplorer™'s buffer reagents are recommended when running hybridization assays with GenoExplorer™ Chips. The reagents have been developed to optimize the stringency and specificity of the assay.

- **Streptavidin-Dye Staining**

The GenoExplorer™ full kit also includes a streptavidin-dye conjugate which is used for the post-hybridization dye staining step. This step is required for RNA labeled with biotin.

Kit Components and Storage Conditions

GenoExplorer™ miRNA Full Kit for 20 or 4 Reactions

Components	20 Reaction Amount	4 Reaction Amount	Storage
Buffer L	140 µl	28 µl	-20º C
Enzyme L	10 µl	2 µl	-20º C
SA-S Dye	20 µl	5 µl	-20º C
Buffer S	2 vials of 1.4 ml each	0.56 ml	-20º C
Array Chip	20 chips	4 chips	+4º C
2x Hyb Buffer	1.4 ml each	280 µl	+4º C
Wash Buffer I (Concentrate)	120 ml	120 ml	+4º C
Wash Buffer II (Concentrate)	50 ml	50 ml	+4º C
Array Layout CD	Web download	Web download	
User's Manual	Web download	Web download	

Shipping and Storage

GenoExplorer™ miRNA Labeling Kit reagents are shipped on dry ice. GenoExplorer™ Biochips and Hybridization Reagents are shipped at room temperature. Components should be stored at temperatures shown in the above table. At proper storage conditions, components are stable for 1 year from the date received. Expiration dates are also noted on product labels.

Safety Warnings and Precautions

For research use only. Not recommended or intended for the diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Consider all chemicals as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Exercise caution to avoid contact with skin or eyes: if contact should occur, wash immediately with water (Material Safety Data Sheet for products is available upon request).

Additional Required Materials

Total RNA containing the small RNA
RNase-free water
Adjustable pipettors
RNase-free tips
RNase-free polypropylene microcentrifuge tubes (0.2, 0.5 or 1.5 ml)
Graduated cylinder
Microcentrifuge
Incubator set at 37º C
Incubator or heating block set at 75º
Heating block at 95º C
Hybridization Station (optional), for automated hybridization (*e.g.*, Genomic Solutions, Tecan) **or**
Coverslips, for manual hybridization (*e.g.*, Erie LifterSlips™ , Product # 22x30I-2-4374)
Slide Chamber, for manual hybridization (*e.g.*, Corning® Microarray Hybridization Chamber, Product #2551)
Incubator or water bath set at 42º C, for manual hybridization
Bottles for storing diluted Wash Buffers
Centrifuge or air blower
Microarray scanner (*e.g.,* Axon, Agilent, Parkard) and image processing software
Several wash containers appropriately sized to the number of chips being used

The GenoExplorer™ full kit is open to a variety of equipment. The examples given are only suggestions rather than specific recommendations. Please contact technical support if you have specific questions.

Related Products from GenoSensor

GenoExplorer™ microRNA Labeling Kit (Cat# 1301)
GenoExplorer™ microRNA Biochips Kit (Cat# 1201C – 1299C)
GenoExplorer™ microRNA Probe Set (Cat# 1401C – 1499C)
GenoExplorer™ Reagents for Hybridization Assay (Cat# 1500's)

GenoExplorer™ Labeling Protocol

General Description

The GenoExplorer™ miRNA Labeling Kit (patent pending) provides a direct end-labeling method. Biotin labels are ligated to the 5' ends of RNA molecules which do not contain a 5'-capped structure. These RNA molecules include rRNA, tRNAs, regulatory small RNAs such as microRNA, siRNA, snRNAs, and other RNA transcripts of yet unknown function. Due to labeling selectivity, this method has resulted in low false positive hybridization signals that are usually caused by mRNA, which are the most highly complex sequences of genome transcripts. This protocol uses directly isolated RNA without RNA target amplification, and ultimately reflects the cellular microRNA molar ratio, thus providing for the reliability of hybridization signals. The streptavidin-conjugated dye stain (SA-S Dye) and the staining buffer (Buffer S) reagents are also included in this kit. Their preparation and use in the post-hybridization staining procedure is described below in the hybridization assay section.

Handling RNA Samples

When working with RNA, always use proper microbiological aseptic techniques. Use RNase- and DNase-free reagents, water, glassware and plasticware. Use non-powdered gloves during all steps of sample labeling, chip hybridization, washing, detection, and scanning.

RNA Preparation

The GenoExplorer™ miRNA Labeling Kit (patent pending) provides an easy and quick way to label microRNAs and other small RNAs. Total RNA isolation (not provided) using traditional methods such as Trizol is recommended. Some commercial kits can be used. Users should be aware of the harvest efficiency for small RNAs when choosing them. Checking with manufacturers is highly recommended. A total RNA starting amount of 5 to 10 micrograms is recommended.

High quality and sufficient amounts of RNA samples is crucial for experiments with microarrays. RNA quality can be evaluated by visualizing the RNA on a gel, as well as by calculating the A_{260}/A_{280} ratio. On a denaturing gel (or on an ordinary agarose gel in denaturing buffer) the RNA should appear as two bright distinct bands that represent the 28S and 18S ribosomal species. The 28S band should be brighter than the 18S band. Tailing of these major bands down the gel, or a background smear behind these bands that gets heavier at lower molecular weights can indicate degradation of the RNA. Degraded RNA will produce high background and low signal intensity microarray results.

miRNA Labeling Procedure

This procedure is used to attach a biotin label to the 5' ends of RNA molecules.

1. Place Buffer L on ice and thaw for 15-20 minutes. Check for any precipitate. If necessary, warm the solution to 37°C and agitate to dissolve the precipitate completely.
   Note: Aliquot is recommended to minimize thaw/freeze cycles
2. Mix Buffer L by vortexing followed by brief centrifugation.
3. Combine reagents according to Table 1 below for a single reaction. Reagents should be combined in an RNAase-free microcentrifuge tube and all reagents should be kept on ice during set up of the reaction. For high accuracy, pipet the viscous Buffer L slowly.

Table 1: miRNA Labeling Reaction Mix

Reagents	Volume (µl)
Buffer L	7.0
RNA (2.5-10µg)	Adjustable
RNAase-free water	Adjustable
Enzyme L	0.5
Total	**20**

4. Mix reaction thoroughly by pipetting up and down several times.
5. Incubate at 20º C for 3 hours.
6. Incubate at 75º C for 20 min to inactivate the enzyme.
7. Store on ice until ready for the hybridization step. The labeled sample can also be stored at -70º C and used later.

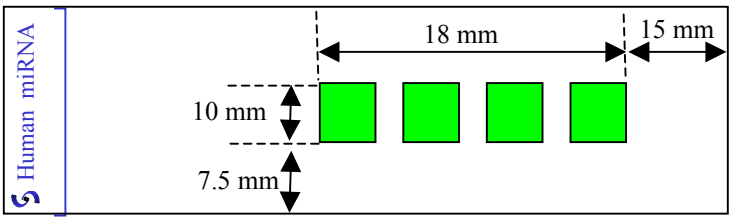
GenoExplorer™ miRNA Biochips Hybridization Assay Protocol

General Description

GenoExplorer™ biochips are built on a 1" x 3" (25 mm x 75 mm) slide. The chips are coated with patented polymers used for DNA probe attachment. The biochips covalently immobilize amine-modified DNA oligonucleotide probes for use in microarray analysis. The chips demonstrate high probe attachment efficiency and accessibility of nucleic acid targets for hybridization.

Figure 1 shows the positions of the label and probe array on the chip. Each array is composed of multiple sub-arrays*. The sub-arrays are positioned lengthwise 15 mm from the end of the chip opposite the label and centered widthwise. For example of human array (Fig 1), sub-arrays are positioned lengthwise 15 mm from the end of the chip opposite the label and widthwise 7.5 mm from the side of the chip. The entire array area covers a length of 18 mm and a width of 10 mm.

Figure 1: Sub-array Positions on the Chips



***Note:** The number of sub-arrays and positioning will vary slightly depending on species, check .gal file for specifics.

Biochip Handling and Assembly

Biochips are stored in a chip container. It is recommended to wear powder-free gloves when handling the biochips. Open the container carefully to avoid dropping chips. Hold the chip at its edges and avoid touching the side of the chip containing the probe array. The probes are on the side of the chip which faces you when reading the chip label correctly (refer to Figure 1). Prepare the chips for hybridization promptly to minimize possible accumulation of dust from the air on to the surface of the arrays (even if some dust can be washed away after post-hybridization washing procedures). Use assembly methods as recommended by hybstation or hybchamber manufacturers. For automated hybridization using a hybstation, orient the chips such that the probe side is up in the chamber and the chip-end opposite the label (where the arrays are located) are next to the sample injection port. Once the hybridization solution is introduced to the chip, avoid any drying of the chips until after the final wash step.

Assay Protocol

Preparation of Wash Buffers and SA-S Dye Stain Solution

1. Prepare Wash Buffer I by adding 1880 ml distilled H_20 to the concentrate. Final volume will be 2000ml.

2. Prepare Wash Buffer II by adding 950 ml distilled H_20 to the concentrate. Final volume will be 1000ml.

Prepare 1:250 dilution of the SA-S dye stain within 15 minutes of staining step

1. For a single sample this dilution should be performed by adding 1 µl of the SA-S to 249 µl of buffer S followed by gentle mixing and brief centrifugation.
2. Keep protected from light to minimize photobleaching of dye.

The following two sections provide guidelines for running the assay either by an automated method or a manual method. The first section describes an automated procedure using a hybstation. The second section describes a manual procedure using LifterSlips™ and a hybchamber.

Assay Using an Automated Method

1. Prepare the labeled RNA sample for hybridization by combining with 2x Hyb buffer and water. The volumes of each component to be added will depend on the volume of the hybridization chamber. Table 2 shows the volumes of each component required for different chamber volumes.

Table 2: Hybridization Reaction Mix

Chamber Volume (µl)	Labeled RNA (µl)	2x Hyb Buffer (µl)	Added H_2O (µl)
40	20	20	0
60	20	30	10
100	20	50	30
120	20	60	40

2. Incubate the sample at 90-95º C for 5 min to denature.
3. Cool sample by spinning down briefly.
4. Inject the sample into the hybridization chamber.
5. Run hybridization at 42º C with agitation for 16 hours (or longer).
6. Run three wash cycles at 25 ºC using diluted Wash Buffer I. Wash time: 20 sec., soak time: 10 sec.
7. Inject the 1:250 diluted SA-S dye stain solution into the hybridization chamber. Incubate at 25ºC for 30 min. Shield the chamber from light to minimize effects of photobleaching of the dye.
8. After the incubation, unload chip from the hybstation and transfer into an appropriately sized container filled with Wash Buffer I, use no less than 20ml Wash Buffer I per chip per wash. Wash chip at room temperature for 5 minutes with periodic, gentle agitation.
9. Transfer chip to a second container with fresh Wash Buffer I. Wash chip again at room temperature for 5 minutes with periodic, gentle agitation.
10. Transfer chip to a third container filled with Wash Buffer II. Wash at room temperature for 30 sec with continuous, gentle agitation.
11. Dry chip by air blower or centrifugation at 600 x *g* for 3 min.

Assay Using a Manual Method

1. Prepare the labeled RNA sample for hybridization by combining with 2x Hyb Buffer and water. The volumes of each component to be added will depend on the volume appropriate for the LifterSlip™. Table 2 shows the volumes of each component for a range of total hybridization volumes.
2. Incubate the sample at 90-95º C for 5 min to denature.
3. Cool sample by spinning down briefly.
4. Prepare the hyb chamber by adding an appropriate volume of the 2x Hyb Buffer to the designated places in the chamber to provide humidity.
5. Place the chip in the chamber with the probe array side up and place the coverslip on top of the array. Refer to Figure 1 to ensure proper orientation of the chip in the chamber and proper placement of the LifterSlip™ over the array position.
6. Position the pipette tip along an open (short) edge of the LifterSlip™, and slowly and carefully pipet the volume of prepared sample under the LifterSlip™, until the array surface underneath is completely covered with the sample. When pipetting, be careful not to form bubbles under the slip. If bubbles appear, you may try to remove them by gently tapping the LifterSlip™ with a pipette tip.
7. Seal the hyb chamber completely and place in an incubator (or water bath) at 42º C.
8. Incubate at 42º C for 16 hours (or longer).
9. After the hybridization incubation, remove from the hyb chamber and transfer into an appropriately sized container filled with Wash Buffer I, use no less than 20ml Wash Buffer I per chip per wash. Wash chip at room temperature for 5 minutes with periodic, gentle agitation. The coverslip should separate from the chip and fall to the bottom of the container with gentle agitation.
10. Transfer to a second container with fresh Wash Buffer I. Wash chip again at room temperature for 5 minutes with periodic, gentle agitation.
11. Transfer to a third container with Wash Buffer II. Wash at room temperature for 30 sec with continuous, gentle agitation.
12. Dry chip by air blower or centrifugation at 600 x *g* for 3 min.
13. Prepare the hyb chamber by adding an appropriate volume of the Buffer S to the designated places in the chamber to provide humidity.
14. Place the chip in the chamber with the probe array side up and place the coverslip on top of the array. Refer to figure 1 to ensure proper orientation of the chip in the chamber and proper placement of the LifterSlip™ over the array position.
15. Position the pipette tip along an open (short) edge of the LifterSlip™, and slowly and carefully pipet the volume of prepared 1:250 diluted SA-S dye stain solution under the LifterSlip™, until the array surface underneath is completely covered with the sample. When pipetting, be careful not to form bubbles under the slip. If bubbles appear, you may try to remove them by gently tapping the LifterSlip™ with a pipette tip.
16. Incubate at 25 ºC for 30 min. Shield the chamber from light to minimize effects of photobleaching of the dye.
17. After the staining incubation, remove chip from the hyb chamber and transfer into a container with Wash Buffer I. Wash chip at room temperature for 5 minutes with periodic, gentle agitation. The coverslip will separate from the chip and fall to the bottom of the container with the gentle agitation.

18. Transfer chip to a second container filled with fresh Wash Buffer I Wash chip at room temperature for 5 minutes with periodic, gentle agitation. .
19. Transfer chip to a third container filled with Wash Buffer II. Wash at room temperature for 30 sec with continuous, gentle agitation.
20. Dry chip by air blower or centrifugation at 600 x *g* for 3 min.

Scanning and Detection

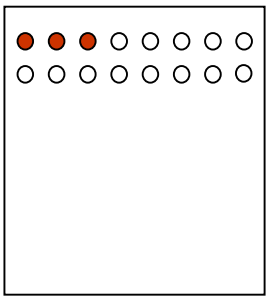
Consult the scanner manufacturer instructions for laser and PMT settings. The SA-S dye stain signal can be detected using the scanner's 635 nm wavelength (Cy5) channel.

When scanning, start from the end of the chip opposite the label.

Using the Grid Files

The .gal web file download provides an array list in .txt file and .gal file formats. Visit http://www.genosensorcorp.com/microRNA_full_kit.html for downloads. Multiple sub-arrays were printed on each chip. The name and location of each chemical probe is listed and labeled in the software. Each chemical was printed in triplicate. Arrays include known microRNA probes as well as both positive and negative controls. Load the .gal files or convert the text file to the correct format that your scanner requires. Since the first chemicals on each sub-array are positive controls, you should see them on the upper-left corner of each sub-array, and they are in triplicate. Move the array grid until it aligns with the positive controls in the upper-left corner (Figure 2). Proceed with data computation after gridding.

Figure 2: Grid and Align the Array



Appendix

Troubleshooting Guide

Poor Hybridization Signal

Poor quality RNA samples	• Use higher quality RNA samples • Use proper laboratory techniques when handling RNA samples • Label and hybridize more sample to microarray
Suboptimal amount of sample applied to microarray	• Label and hybridize more sample to microarray
Improper detection strategy	• Verify that detection instrumentation is compatible with detection reagents • Adjust detection settings
Signal lost by exposure to light, environmental conditions	• Minimize exposure to light
Poor biotin/ SA-S dye detection	• Ensure that the samples have been purified and quantified properly before labeling • Ensure that the kit components have been stored properly • Ensure that the detection solution was prepared and stored properly
Hybridization signal “stripped” from microarray	• Ensure correct temperatures are used for each of the process steps.
Suboptimal hybridization time	• Extend hybridization time

High Background

Excess detection reagents remaining on microarray	• Do not allow chips to dry out during hybridization protocol • Do not touch microarray directly or forcibly remove coverslip at any time
Excess sample applied to microarray	• Quantify the amount of labeled sample and use less in hybridization
Ink or marker used to identify microarray	• Avoid using markers or ink to identify chip; use a diamond scribe pen

Dust

Dust on microarray	• Avoid dust from environment • Minimize exposure of the microarray to the air • Use filtered distilled water for final wash

Technical Service

For more information or technical assistance, please call, write, fax, or email.

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