Signosis
BioSignal Capture

MiRNA Northern Blot Assay Kit

Catalog Number NB-0001 **(For Research Use Only)**

Introduction

Expression of miRNAs is highly regulated and tissue-specific. Many of miRNAs are differentially expressed in human cancers. Microarray profiling of miRNA expression leads to identification of differentially expressed miRNA in cancers. MiRNA expression, tissue distribution, and microarray data validation often need northern blot analysis, with which the difference can be directly visualized, mature miRNA can be differentiated from its precursor, and the expression level of miRNA can be compared with internal controls such as U6 RNA. Signosis miRNA northern blot assay kits provide a non-radioactive, simple, and highly sensitive tool to conduct the assay. These kits contain all the components needed for northern blot analysis. More importantly, these kits do not need the use of isotope and probe preparation. Reagents for RNA transfer, hybridization, and detection are included in the kits.

Materials provided with the kit

1. 30 µl Ready-to-use small sizes of molecular standards (20 nt & 60 nt) (-20^{o}C)
2. 90 µl Gel loading buffer (RT)
3. Two 15% TBE urea-gel (4 ^{o}C)
4. Two Membranes (RT)
5. 35ml 1x NB Hybridization buffer (RT)
6. 40ml 5x NB Hybridization wash buffer (RT)
7. 60ml Blocking buffer (RT)
8. 60 µl Streptavidin-HRP conjugate (4 ^{o}C)
9. 50ml 5x Detection wash buffer (RT)
10. 3.6 ml Substrate A (4 ^{o}C)
11. 3.6 ml Substrate B (4 ^{o}C)

Materials and equipment are needed

1. Biotin labeled miRNA probe (MP-0XXX, Signosis)
2. TBE
3. Bio-Rad gel apparatus
4. Power supplies
5. Stratagene UV cross-linker
6. Hybridization oven
7. Hybridization tubes
8. Shaker
9. Imaging system or X-ray film

Reagent preparation before experiment

- Dilute the 5x Hybridization wash buffer and 5x Detection washing buffer to 1x buffer
- 1x Hybridization wash buffer:
  30ml 5x Hybridization wash buffer
  120ml ddH2O
- 1x Detection wash buffer:
  40ml 5x Detection wash buffer
  160 ml ddH2O
- Prewarm 1x NB Hybridization buffer and 1x Hybridization wash buffer.
  In order to be dissolved completely, the buffers need to be warmed up for 5 to 16 hours at 45 ^{o}C, and may also need to be stirred with a 5ml or 10ml pipette periodically, until the buffers are clear without any visible precipitation.

Assay Procedure

1. Gel electrophoresis

(1) Remove the comb and the bottom plastic sealer from the gel.
(2) Assemble the gel and pre-run at 60V for about 30 min using pre-chilled 0.5X TBE as the running buffer.
(3) While pre-running the gel, prepare RNA samples by mixing 3 µl of RNA loading buffer with 7 µl (5 µg) of total RNA, heating at 70^{o}C for 5 min and chill on ice.
(4) Rinse individual wells by pipetting the buffer up and down before loading RNA samples.
(5) Carefully load 10 µl RNA sample onto one well of 15% pre-run urea-polyacrylamide gel. Load 5 µl molecular standards next to the RNA sample.
Note: Different RNA samples can be loaded onto the gel for the detection with a single miRNA probe or a same RNA sample can be loaded onto different wells for the hybridization with different miRNA probes (see Table 1 for recommended arrangement). An empty well between two different hybridization groups is recommended for easy cutting after RNA transfer.
(6) Run at 60V until bromophenol blue reaches approximately 3 cm away from the bottom of the gel.

2. Transfer

(1) Dissemble the gel cast and remove one of the plates from the gel.
(2) Transfer the gel to a glass tray filled with 0.5X TBE buffer.
(3) Soak the membrane and filter paper in 0.5X TBE.

(4) Assemble the transfer unit in the following order on the black side of cassette: one fiber pad, one piece of filter paper, gel, membrane and one piece of filter paper, one fiber pad.
(5) Make sure the gel at negative side and membrane at positive side and transfer cassette to BioRad Trans-Blot Cell and fill with pre-chilled 0.5xTBE.
(6) Transfer at 60V at for l hr in a cold room or put the tank on ice within an ice basket.
(7) After transfer, RNA is immobilized with Stratagene UV cross-linker.
(8) Dry at 42^{o}C for 15 min.

3. Hybridization

(1) Put the membrane into the hybridization tube (Corning 50 ml disposable tube recommended).
(2) Soak the membrane with dH_2O, and then discard the dH_2O.
(3) Add pre-warmed 4 ml of NB hybridization buffer (pre-warmed to 42^{o}C) into the bottle.
(4) Rotate at 42^{o} C for 30 min
(5) Replace the buffer with 4 ml of fresh NB hybridization solution pre-warmed at 42^{o}C.
(6) Add 10 µl of miRNA probe and rotate at 42^{o}C overnight.
(7) Rinse the membrane in the bottle with 20ml of 1xNB hybridization wash buffer.
(8) Add 20 ml of NB hybridization wash buffer and rotate at 42^{o}C for 30 min.

4. Detection

(1) Using forceps, transfer the membrane from the hybridization tube to a container (an empty 200 µl pipette tips box). Each box could have one full membrane or two half size of the membranes (don't overlap the membrane during all of the following incubation steps.
(2) Rinse the membrane with 10 ml of 1X Detection wash buffer.
(3) Block the membrane with 15 ml of Blocking buffer for 30 minutes at room temperature with moderate shaking.
(4) Dilute 15 µl of Streptavidin-HRP conjugate with 1 ml of the 1X Blocking buffer and transfer to the container. Do not add HRP diluted solution directly onto the membrane.
(5) Continue shaking the membrane for 45 min at room temperature.
(6) Decant the Blocking buffer and wash three times at room temperature with 15 ml of 1x Detection washing buffer, 10 minutes each wash.
(7) Mix equal amounts of Substrate A and B. Place the membrane on the bottom side of detection sheet on a flat surface, and overlay the membrane with 1.8 ml of substrate solution, ensuring that the substrate is evenly distributed over the membrane. Gently place the top side of detection sheet over the membrane ensure that the substrates cover the entire surface of the membrane, without trapping air bubbles on the membrane. Incubate at room temperature for 5 minutes.
(8) Remove excess substrate by gently applying pressure over the top sheet using a paper towel. Expose the membranes using either Hyperfilm (2-10 min) or a chemiluminescence imaging system (i.e., FluorChem imager from Alpha Innotech). With either method, experiment with different exposure times.

Sample of miRNA Northern blotting analysis

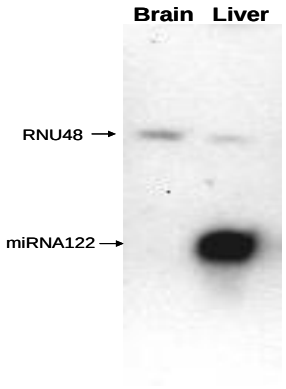

Table 1

One Full Size Gel	Membrane Size	# Probes	Hybridization Tube	Detection	Substrate
1 standard, 14 RNA samples	Full size membrane	1	1	1 membrane per container	1.8 ml per pieces
1 standard, 6 samples (two duplicates with one blank well in between)	1/2 size membrane	2	2	2 membranes per container	1 ml per pieces
1 standard, 2 RNA samples (three duplicates with one blank wells in between each duplicate)	1/3 size membrane	3	3	2 membranes per container	0.75 ml per pieces