Signosis
BioSignal Capture

# Cancer MiR-21 Northern Blot Assay Kit

**Catalog Number NB-0011** **(For Research Use Only)**

## Introduction

miR-21 is overexpressed in a wide variety of cancers. It is believed to act as an oncogene by downregulating the expression of many tumor suppressors, including programmed cell death 4 (PDCD4) (1), tumor suppressor Pdcd4 (2), the PTEN tumor suppressor gene (3), the tumor suppressor gene tropomyosin 1 (TPM1) (4). miR-21 also found to be an important regulator of cancer-cell survival. Differently from the survival pathway mediated by IL-6/Stat3 activation through expression of antiapoptotic proteins, miR-21 is upregulated by Stat3, which mediates the suppression of apoptosis possibly through the inhibition of TPM1 and/or other proteins.

## Materials provided with the kit

1. 30 µl Ready-to-use small sizes of molecular standards (20 nt & 60 nt) (-20°C)
2. 90 µl Gel loading buffer (RT)
3. 2 Membranes (RT)
4. 35ml 1x NB Hybridization buffer (RT)
5. 40ml 5x NB wash buffer (RT)
6. 60ml Blocking buffer (RT)
7. 60 µl Streptavidin-HRP conjugate (4 °C)
8. 50ml 5x Detection wash buffer (RT)
9. 3.6 ml Substrate A (4 °C)
10. 3.6 ml Substrate B (4 °C)
11. miR-21 probe

## Materials and equipment are needed

1. TBE
2. Bio-Rad gel apparatus
3. Power supplies
4. Stratagene UV cross-linker
5. Hybridization oven
6. Hybridization tubes
7. Shaker
8. Imaging system or X-ray film

## Reagent preparation before experiment

- Dilute the 5x Hybridization washing buffer and 5x Detection washing buffer to 1x buffer
- 1x Hybridization washing buffer:
  30ml 5x Hybridization washing buffer
  120ml ddH2O
- 1x Detection washing buffer:
  40ml 5x Detection washing buffer
  160 ml ddH2O
- Prewarm 1x Hybridization buffer, 1x Hybridization wash buffer at 42ºC for 2 hour or until the buffers are clear without visible precipitation before using.

## 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°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

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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 1 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 $dH_2O$.
(3) Add pre-warmed 4 ml of NB hybridization solution (pre-warmed to 42$^o$C ) into the bottle.
(4) Rotate at 42$^o$ C for 30 min
(5) Replace the solution 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 NB wash buffer.
(8) Add 20 ml of NB 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 20 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 ECL (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**

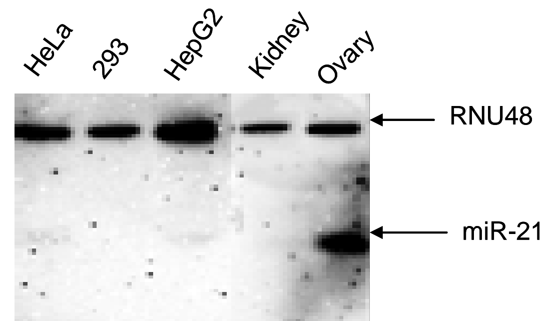


**Table 1**

| One Full Size Gel | Membrane Size | # Probes | Hybridization Tube | Detection | Substrate |
|---|---|---|---|---|---|
| 1 standard, 14 RNA samples | Full size membrane (2 pieces) | 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 (4 pieces) | 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 (6 pieces) | 3 | 3 | 2 membranes per container | 0.75 ml per pieces |

## References

(1) Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2007, 283:1026-1033

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(3) Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007, 133:647-658.

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(5) Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res. 2005, 65:6029-6033.