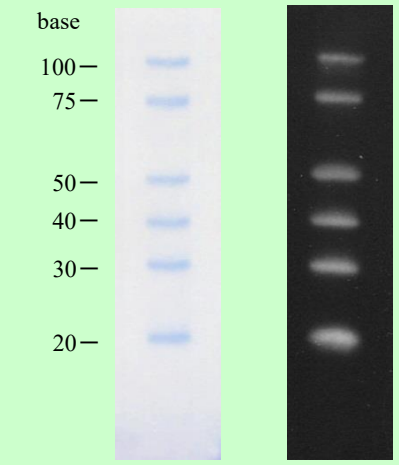
Product Name : DynaMarker DIG Labeled Blue Color Marker for Small RNA
(Previous name : DynaMarker Colored DIG Marker for Small RNA)

Code No. : DM270
Range : 20 - 100 bases
Size : 125 µl (5 µl × 25 loadings)
Storage : store at -20 °C

Description :
The DynaMarker DIG Labeled Blue Color Marker for Small RNA consists of colored (Blue) and DIG labeled six single-strand nucleic acids, the apparent molecular weights of which are 20, 30, 40, 50, 75 and 100 bases of RNAs. This marker is suitable for monitoring denaturing polyacrylamide gel electrophoresis and for immunodetection with anti-DIG antibody.
The apparent sizes of bands in DynaMarker DIG Labeled Blue Color Marker for Small RNA are in excellent agreement with sizes of non-stained RNAs, 20, 30, 40, 50, 75 and 100 bases in length (about 95 % accuracy, see Table 1).
The DynaMarker DIG Labeled Blue Color Marker for Small RNA is supplied in a ready-to-use mixture and doesn't require heating or addition of a denaturing agent before use.



DynaMarker DIG Labeled Blue Color Marker for Small RNA

Figure 1. Electrophoresis profile of DynaMarker DIG Labeled Blue Color Marker for Small RNA (5 µl) on 15 % polyacrylamide - 7.5 M urea gel / 1 × TBE buffer as running buffer.

Storage buffer :
2 mM Tris-HCl (pH8.0), 8mM EDTA, 78 % Formamide

Quality Control :
After 24-hrs incubation of the DynaMarker DIG Labeled Blue Color Marker for Small RNA at 37 °C, no visible degradation of the marker is observed in 15 % polyacrylamide - 7.5 M urea gel electrophoresis.

Recommended loading volumes : 5 - 10 µl

Electrophoresis condition :
Be sure to use this marker on 10 - 15% acrylamide - Urea /1 × TBE gel and 1 × TBE as running buffer. Under other conditions, the bands cannot be separated correctly.

Note :
For accurate electrophoretic determination of molecular weights, the DynaMarker Small RNA II (code # DM192) or DynaMarker Small RNA II Easy Load (code # DM197) should be used.

BioDynamics Laboratory Inc.
www.biodynamics.co.jp

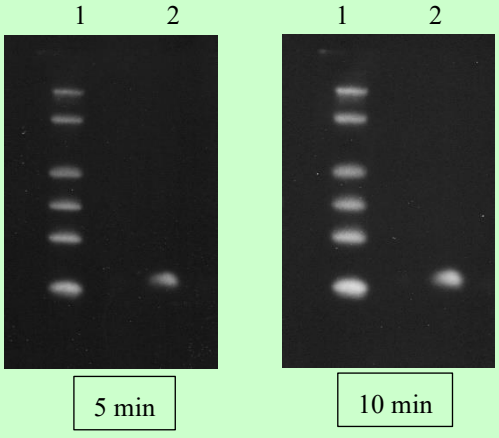
DynaMarker Small RNA II + 75 base RNA	acrylamide concentration 10 %	15 %
100 base	**103.9 %**	**101.3**
75*	**104.8**	**100.6**
50	**102.5**	**101.6**
40	**103.0**	**100.5**
30	**99.6**	**104.5**
20	**100.0**	**102.8**

Table 1. This shows apparent molecular weights compared with the DynaMarker Small RNA II, and suitable acrylamide concentrations for electrophoresis of the DynaMarker DIG Labeled Blue Color Marker for Small RNA.

(* 75 base RNA is from a newly synthesized RNA. A 75 base RNA is not included in DynaMarker Small RNA II.)

Sensitivity :

This marker is suitable for chemiluminescent (e.g. CDP-*star* *1) immunoassay. The sensitivity depends on the length of exposure to high speed instant film (e.g. FP-3000B *2), X-ray film or imaging instrument. The following figure shows an example.



Film: Fujifilm FP-3000B
instant black & white film
Exposure: 5 - 10 min at room temperature

Lane 1	This Marker (5 µl)
Lane 2	MCF-7 total RNA (5 µg)

15 % acrylamide - 7.5 M Urea / 1 × TBE

Figure 2. Detection of DynaMarker DIG Labeled Blue Color Marker for Small RNA and hsa-miR-21.

5 µl of DynaMarker DIG Labeled Blue Color Marker for Small RNA and 5 µg of MCF-7 total RNA were blotted onto nylon membrane, and the hsa-miR-21 was hybridized with the DIG labeled DNA probe (2.5 nM). This marker and the DIG labeled DNA probe were detected with anti-DIG-AP antibody and CDP-*star*.

*1: CDP-*star* is a trademark of Tropix, Inc.

*2: FP-3000B is a product of Fujifilm Corp.

Recommended usage :

The DynaMarker DIG Labeled Blue Color Marker for Small RNA is suitable for monitoring denaturing acrylamide gel electrophoresis and blotting onto membrane. One example is shown below:

・Electrophoresis and blotting of DynaMarker DIG Labeled Blue Color Marker for Small RNA

1) Preparation of 12.5 % polyacrylamide - 7.5 M urea gel

40 % acrylamide : bis solution	6.25 ml
Urea	9.0 g
10 × TBE	2.0 ml
H2O	to 20 ml

After urea is dissolved completely, add 20 µl of TEMED and 100 µl of 10 % ammonium persulfate. Mix quickly then pour the gel into the mold of a vertical gel apparatus.

2) Loading and electrophoresis.

Thaw the DynaMarker DIG Labeled Blue Color Marker for Small RNA completely before use. Load the denatured RNA sample and 5 µl of DynaMarker DIG Labeled Blue Color Marker for Small RNA into a well and run the gel using 1 × TBE electrophoresis buffer at 200 V.

3) Transfer the DynaMarker DIG Labeled Blue Color Marker for Small RNA and RNA from gel to membrane (Figure 3).

3-1) Cut a piece of positive charged nylon membrane slightly larger than the gel. Soak the membrane and four sheets of blotting paper of appropriate size in 1 × TBE buffer.

3-2) Place two sheets of blotting paper on the anode platform of the transfer cell.

3-3) Place the membrane on top of the blotting paper.

3-4) Transfer the gel from the glass plate to the top of the membrane and press out any air bubbles. (*Make sure that there are no air bubbles between the membrane and the gel.)

3-5) Place another two sheets of blotting paper onto the gel and set the cathode assembly.

3-6) Transfer for 30 - 60 min at 2 mA/cm^2.

3-7) After ensuring the marker has transferred successfully onto the membrane, remove both paper and gel. Rinse the membrane in 2 × SSC.

3-8) Fix the RNA to the membrane with a UV crosslinker.

3-9) Carry out northern hybridization (Figure 4).

References:

- Joseph Sambrook, and David W. Russell (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press.
- Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J. G. Seidman, John A. Smith, and Kevin Struhl (1994－) Current Protocols in Molecular Biology, John Wiley & Sons, Inc.
- Sang Woo Kim, Zhihua Li, Patrick S. Moore, A. Paula Monaghan, Yuan Chang, Mark Nichols and Bino John (2010) A sensitive non-radioactive northern blot method to detect small RNAs. Nucleic Acids Research. **38**(7): e98

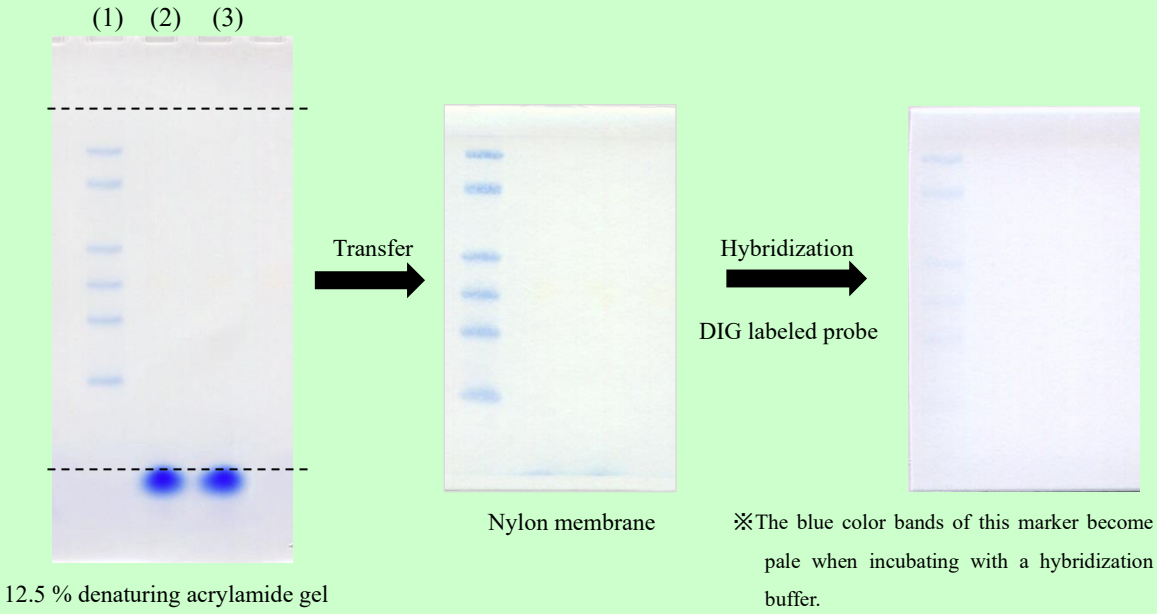


Figure 3. **Left:** Electrophoresis profile of (1) 5 µl of DynaMarker DIG Labeled Blue Color Marker for Small RNA, (2) 5 µg of Human Breast total RNA and (3) 5 µg of Breast Adenocarcinoma (MCF-7) total RNA on 12.5 % polyacrylamide - 7.5 M urea gel / 1 × TBE buffer as running buffer.
Center: Blotting of (1) - (3) onto nylon membrane.
Right: Hybridization with the DIG labeled DNA probes of hsa-miR-21 or hsa-miR-16 in a hybridization buffer.

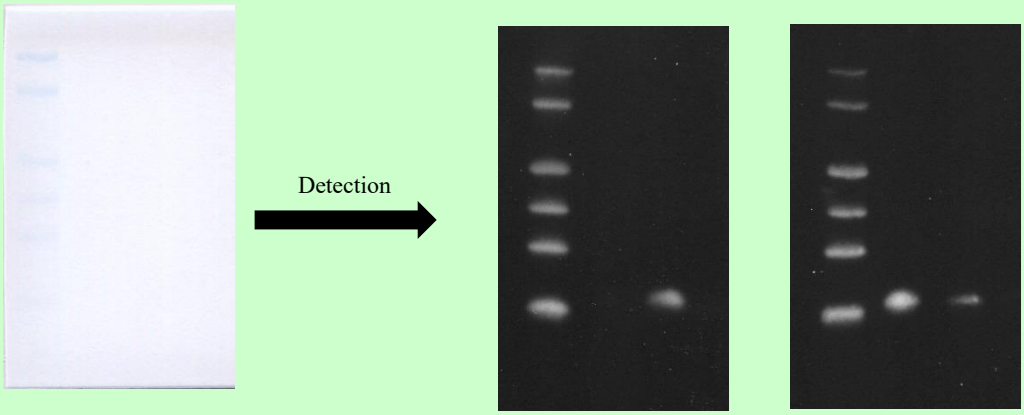


Figure 4. Detection of hsa-miR-21 and hsa-miR-16
The each DIG labeled DNA probes were detected with anti-DIG-AP antibody and chemiluminescent AP-substrate.