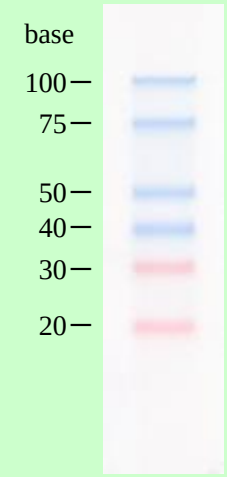
PRODUCT INFOMATION

Product Name : DynaMarker® Prestain Marker for Small RNA Plus
Code No. : DM253
Range : 100 - 20 base
Size : 150 µl (30 loadings)
Storage : store at -20 ℃

Description :
miRNA is known as a non-coding small RNA, involved in many biological events. The miRNA is derived from precursor miRNA (pre-miRNA, about 100 nt) which is cleaved by the RNase III enzyme Drosha from pri-miRNA. The DynaMarker® Prestain Marker for Small RNA Plus consists of six prestained single-strand (blue and red) nucleic acids (apparent molecular weights are 100, 75, 50, 40, 30 and 20 bases) and it is visible during electrophoresis. The DynaMarker® Prestain Marker for Small RNA Plus is suitable for monitoring denaturing polyacrylamide gel electrophoresis and blotting onto membranes. The apparent sizes of bands in DynaMarker® Prestain Marker for Small RNA Plus are in excellent agreement with sizes of non-stained RNAs, 100, 75, 50, 40, 30 and 20 bases in length (about 95 % accuracy, see table 1 and figure 2). The DynaMarker® Prestain Marker for Small RNA Plus is supplied in a ready-to-use mixture and doesn't require heating or addition of a denaturing agent before use.



DynaMarker® Prestain Marker for Small RNA Plus

Figure 1. Electrophoresis profile of DynaMarker® Prestain Marker for Small RNA Plus (5 µl) on 10 % polyacrylamide – 7.5 M urea gel / 1 × TBE buffer as running buffer.

Storage buffer :
2 mM Tris-HCl (pH 8.0), 8 mM EDTA, 78 % Formamide

Quality Control :
After 24-hrs incubation of the DynaMarker® Prestain Marker for Small RNA Plus at 37 ℃, no visible degradation of the marker is observed in 10 % polyacrylamide – 7.5 M urea gel electrophoresis.

Recommended loading volumes :

Comb	Load volume
4-10 mm	5-10 µl
>10 mm	>10 µl

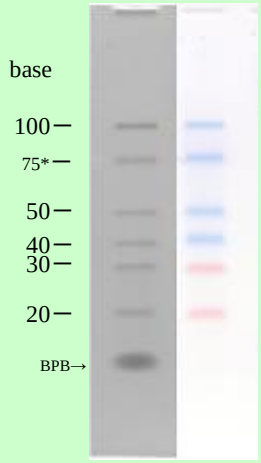
Note :
- For accurate electrophoretic determination of molecular weights, the DynaMarker® Small RNA II (code # DM192) or DynaMarker® Small RNA II Easy Load (code # DM197) should be used.
- A migration of the DynaMarker® Prestain Marker for Small RNA Plus is optimized to use 10 – 15 % acrylamide gel electrophoresis (see table 1).
- This product is not for agarose gel electrophoresis.

acrylamide concentration (condition: acrylamide:bis = 29:1, 1× TBE)

DynaMarker Small RNA II + 75 base RNA	5.0 %	7.5 %	10 %	12.5 %	15 %	17.5 %	20 %
100 base	**105.6 %**	**105.6**	**101.6**	**98.4**	**97.2**	**93.6**	**92.6**
75*	**106.2**	**104.7**	**103.5**	**99.5**	**98.5**	**94.7**	**92.4**
50	**101.4**	**101.4**	**101.1**	**98.7**	**97.5**	**95.0**	**92.2**
40	**103.1**	**102.0**	**103.2**	**100.8**	**100.0**	**97.4**	**93.9**
30	**91.0**	**96.9**	**98.2**	**98.9**	**99.2**	**99.5**	**98.8**
20	**89.8**	**95.8**	**98.2**	**100.3**	**101.6**	**101.4**	**101.4**

Table 1. This shows apparent molecular weights compared with the DynaMarker® Small RNA II, and suitable acrylamide concentrations for electrophoresis of the DynaMarker® Prestain Marker for Small RNA Plus.
■: Recommend ■: Possible

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



10 % polyacrylamide – 7.5 M urea gel

Figure 2. Electrophoresis profile of DynaMarker® Small RNA II (left) and DynaMarker® Prestain Marker for Small RNA Plus (right).

Recommended usage :

The DynaMarker® Prestain Marker for Small RNA Plus is suitable for monitoring denaturing acrylamide gel electrophoresis and blotting onto membrane. One example is shown below:

・Electrophoresis and blotting of DynaMarker® Prestain Marker for Small RNA Plus

1) Preparation of 10 % polyacrylamide – 7.5 M urea gel

40 % acrylamide : bis solution	5.0 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 160 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® Prestain Marker for Small RNA Plus completely before use. Load the denatured RNA sample and 5 µl of DynaMarker® Prestain Marker for Small RNA Plus into a well and run the gel using 1 × TBE electrophoresis buffer at 20 – 40 V / cm.

3) Transfer the DynaMarker® Prestain Marker for Small RNA Plus 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 0.5 × 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 300 mA.

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) Cut off the marker lane.

3-10) Carry out northern hybridization.

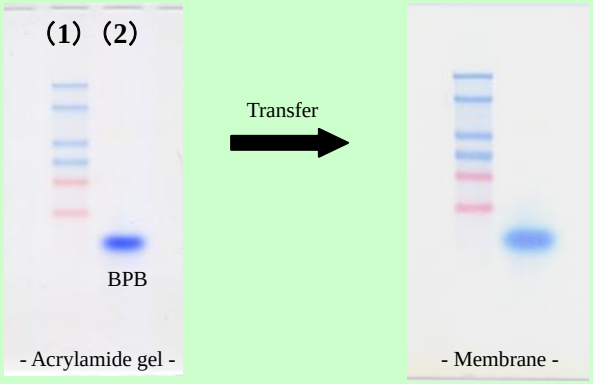


Figure 3. Left : Electrophoresis profile of (1) DynaMarker® Prestain Marker for Small RNA Plus and (2) RNA sample on 10 % polyacrylamide – 7.5 M urea gel / 1 × TBE buffer as running buffer.
Right : Blotting of (1) and (2) onto nylon membrane.

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.