

PRODUCT INFORMATION

Product Name : DynaMarker® Prestain Marker for Small RNA Plus

Code No. : DM253S

Range : 100 - 20 base

Size : 75 µl (15 loadings)

Storage : store at -20 °C

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.

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 °C, 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).



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.

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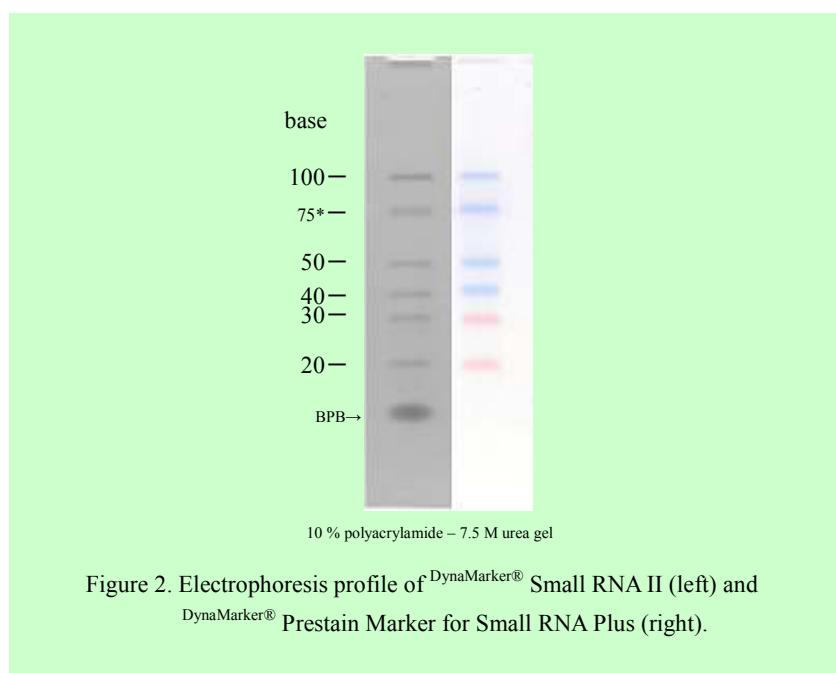
		acrylamide concentration						
		5.0 %	7.5 %	10 %	12.5 %	15 %	17.5 %	20 %
DynaMarker [®] Small RNA II + 75 base RNA	100 base	105.6 %	105.6	101.6	98.4	97.2	93.6	92.6
	75*	106.2	104.7	103.5	99.5	98.5	94.7	92.4
	50	101.4	101.4	101.1	98.7	97.5	95.0	92.2
	40	103.1	102.0	103.2	100.8	100.0	97.4	93.9
	30	91.0	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

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

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.)



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

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40 % acrylamide : bis solution	5.0 ml
Urea	9.0 g
10 × TBE	2.0 ml
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H ₂ O	to 20 ml

After urea is dissolved completely, add 20 μ l of TEMED and 160 μ l 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 μ l 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.

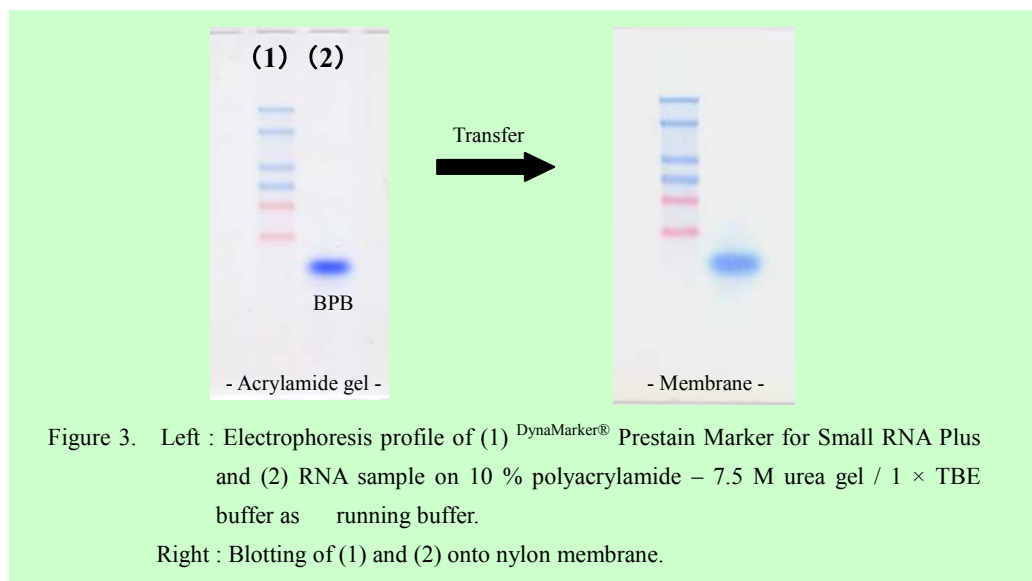


Figure 3. Left : Electrophoresis profile of (1) ^{DynaMarker®} Prestain Marker for Small RNA Plus and (2) RNA sample on 10 % polyacrylamide – 7.5 M urea gel / 1 × TBE buffer as running buffer.
Right : Blotting of (1) and (2) onto nylon membrane.

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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.