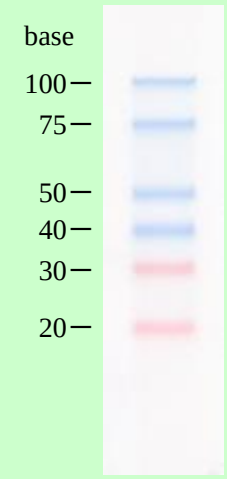
Product Name : DynaMarker Prestain Marker for Small RNA Plus
Code No. : DM253S
Range : 20 - 100 bases
Size : 75 µl (15 loadings)
Storage : store at -20 ℃

Description :
The DynaMarker Prestain Marker for Small RNA Plus consists of six prestained single-strand (blue and red) nucleic acids (apparent molecular weights are 20, 30, 40, 50, 75 and 100 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, 20, 30, 40, 50, 75 and 100 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.

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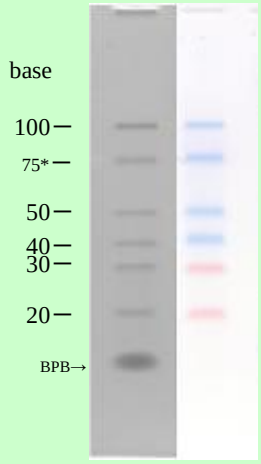
acrylamide concentration (condition: acrylamide:bis = 29:1, 1× TBE)

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.

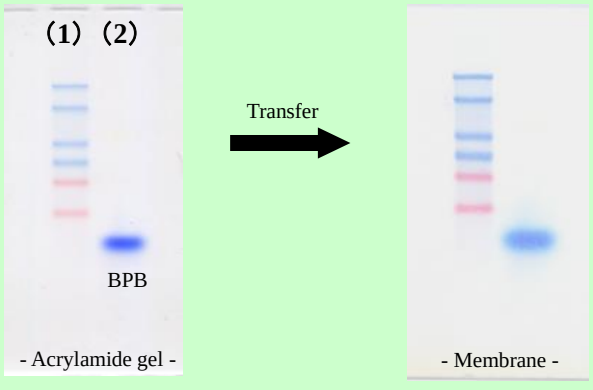


Figure 3. Left : Electrophoresis profile of (1) DynaMarker Prestain Marker for Small RNA Plus and (2) RNA sample on 10 % polyacrylamide – 7.5 M urea gel / 1 × TBE buffer as running buffer.
Right : Blotting of (1) and (2) onto nylon membrane.

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.