

PRODUCT INFORMATION

Product Name : ^{DynaMarker®} Prestain Marker for Small RNA

Code No. : DM250

Range : 50-20 base

Size : 150 µl (30 loadings)

Storage : store at -20 °C

Stability : 6 months

Description :

The ^{DynaMarker®} Prestain Marker for Small RNA consist of four prestained single-strand (blue and red) nucleic acids (apparent molecular weights are 50, 40, 30 and 20 bases) and it is visible during electrophoresis. The ^{DynaMarker®} Prestain Marker for Small RNA is suitable for monitoring denaturing acrylamide gel electrophoresis and blotting onto membranes. The apparent size of ^{DynaMarker®} Prestain Marker for Small RNA are in excellent agreement with sizes of non-stained RNAs, 50, 40, 30 and 20 bases in length (about 95 % accuracy, see table 1 and figure 2). The ^{DynaMarker®} Prestain 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.

Storage buffer :

10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 70 % Formamide

Quality Control :

After 24 hrs incubation of the ^{DynaMarker®} Prestain Marker for Small RNA at 37 °C, no visible degradation of the marker is observed in 12.5 % acrylamide – 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 (code # DM192) or ^{DynaMarker®} Small RNA Easy Load (code # DM197) should be used.
- A migration of the ^{DynaMarker®} Prestain Marker for Small RNA has been optimized to use 10 – 20 % of acrylamide gel concentration (see table 1).
- We recommend marking the band positions on the blotted membrane with a pencil before hybridization as the marker on the membrane pales to some degree during long-term hybridization.



^{DynaMarker®} Prestain Marker for Small RNA

Figure 1. Electrophoresis profile of ^{DynaMarker®} Prestain Marker for Small RNA (5 µl) on 12.5 % acrylamide – 7.5 M urea gel / 1 × TBE buffer as running buffer.

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		concentration of acrylamide		
		10 %	15 %	20 %
DynaMarker Small RNA	50 base	101 %	98	97
	40	104	98	94
	30	102	101	100
	20	105	104	106

Table 1. Apparent molecular weights compared to the DynaMarker® Small RNA

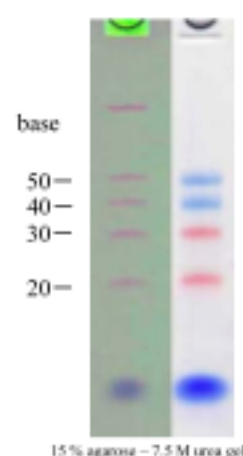


Figure 2. Electrophoresis profile of DynaMarker® Small RNA (left) and DynaMarker® Prestain Marker for Small RNA (right).

Recommended usage :

The DynaMarker® Prestain 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® Prestain Marker for Small RNA

1) Preparation of 12.5 % acrylamide – 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 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 completely before use. Load the denatured RNA sample and 5 µl of DynaMarker® Prestain Marker for Small RNA into a well and run the gel using 1 x TBE electrophoresis buffer at 20–40 V / cm.

3) Transfer the DynaMarker® Prestain Marker for Small RNA and RNA from gel to membrane

3-1) Cut a piece of nylon membrane (e.g. Hybond N+ / GE-Healthcare Bioscience inc.) slightly larger than the gel. Soak the membrane and four sheets of blotting paper of appropriate size in 1x TBE buffer.

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

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- 3-3) Transfer the gel from the glass plate to the top of the membrane and press out any air bubbles.
- 3-4) Place another two sheets of blotting paper onto the gel and set the cathode assembly.
- 3-5) Transfer for 30min – 1hr at 300mA.
- 3-6) After ensuring the marker has transferred successfully onto the membrane, remove both paper and gel. Rinse the membrane in $2 \times$ SSC
- 3-7) Fix the RNA to the membrane with a UV-crosslinker
- 3-8) Mark the band position on the membrane using a pencil, and cut off the pre-stained marker lane. (figure 3)
- 3-9) Carry out northern hybridization.

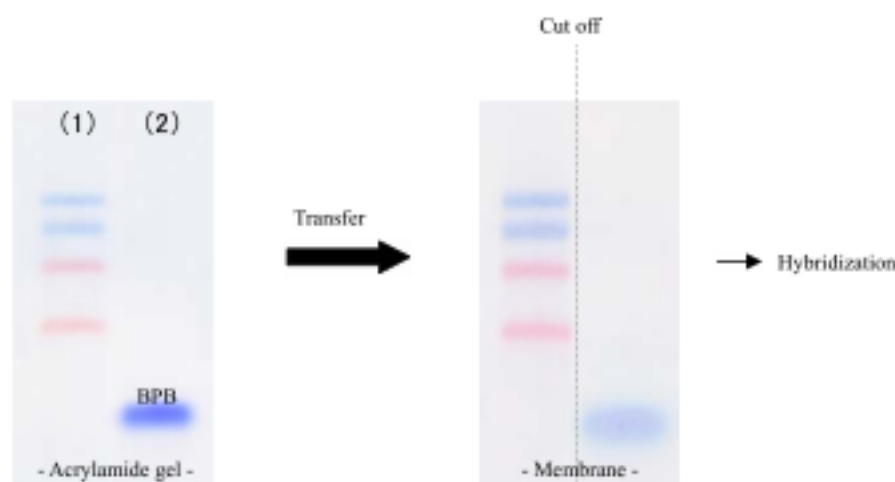


Figure 3. Left : Electrophoresis profile of (1) ^{DynaMaker} Prestain Marker for Small RNA and (2) RNA sample on 12.5 % acrylamide – 7.5 M urea gel / $1 \times$ TBE buffer as running buffer.

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.