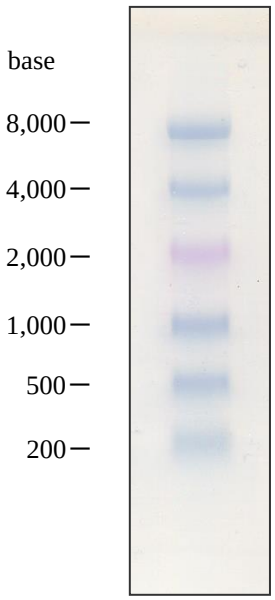
PRODUCT INFOMATION

Product Name: DynaMarker® Prestain Marker for RNA High
Code No. : DM260S
Range: 200-8,000 base
Size: 90 µl (15 loadings)
Storage: store at -80 ℃

Description:

The DynaMarker® Prestain Marker for RNA High is a visible molecular weight marker for ssRNA, consisting of six colored (blue and purple) nucleic acids. The six colored bands (apparent molecular weights are 200, 500, 1,000, 2,000, 4,000 and 8,000 bases) are suitable for monitoring denaturing agarose gel electrophoresis and blotting onto membranes. The DynaMarker® Prestain Marker for RNA High shows the same mobility as that of the DynaMarker® RNA High (code # DM160) on denaturing agarose gel electrophoresis (>90 % accuracy, see table 1 and figure 2). The DynaMarker® Prestain Marker for RNA High is supplied in a ready-to-use mixture without requiring heating or the addition of a denaturing agent before use.



DynaMarker® Prestain Marker for RNA High

Figure 1: Electrophoresis profile of DynaMarker® Prestain Marker for RNA High (6 µl) on 1 % agarose – 2.2 M forumaldehyde gel / 1 × MOPS buffer as running buffer.

Storage buffer:

40 mM MOPS (pH 7.0), 10 mM AcONa, 1 mM EDTA･2Na, 10 % Glycerol

Quality Control:

After 24 hrs incubation of the DynaMarker® Prestain Marker for RNA High at 37 ℃, no visible degradation of the marker is observed in 1 % agarose – 2.2 M formaldehyde gel electrophoresis.

Recommended loading volumes:

Comb	Load volume
4 ~ 6 mm	4 ~ 6 µl
>6 mm	>6 µl

Note:

- For accurate electrophoretic determination of molecular weights, the DynaMarker® RNA High (code # DM160) or DynaMarker® RNA Easy Measurement N (code # DM170) should be used.
- The migration of the DynaMarker® Prestain Marker for RNA High has been optimized to use 0.8 ― 1.5 % of agarose gel concentration (see table 1).
- Particularly avoid freeze – thaw cycle.
- It is not possible to use for acrylamide gel electrophoresis.

concentration of agarose

		0.8 %	1.0 %	1.5 %
RNA High	8000 base	96.5	94.8	90.4
	4000	98.5	100.0	92.2
	2000	104.4	102.8	101.6
	1000	103.4	103.2	101.4
	500	106.4	102.0	103.1
	200	102.7	100.0	108.7

Table 1: Apparent molecular weights as a percentage compared to the DynaMarker® RNA High (DM160).

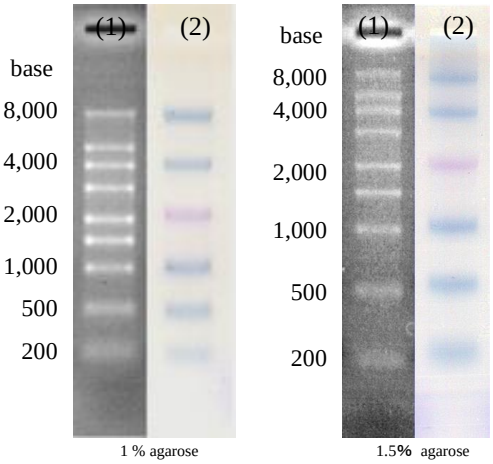


Figure 2: Electrophoresis profile of DynaMarker® RNA High (1) and DynaMarker® Prestain Marker for RNA High (2) on 1 % and 1.5 % agarose.

Recommended usage:

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

▪Electrophoresis and blotting of DynaMarker® Prestain Marker for RNA High

1) Preparation of 0.8 % agarose – 2.2 M formaldehyde gel

Agarose	0.8 g
10 × MOPS	10 ml
deionized formaldehyde	18 ml
RNase free water	72 ml
total	100 ml

Dissolve the agarose by boiling in a microwave oven. Cool the solution to 55 ℃ and add 10 ml of 10 × MOPS buffer and 18 ml of deionized formaldehyde. In a fumehood, cast an agarose gel with slots formed by a 4~6 mm comb. Remove the comb, place the gel in the gel tank, and add sufficient 1 × MOPS running buffer to cover to a depth of ~1 mm.

2) Loading and electrophoresis.

Thaw the DynaMarker® Prestain Marker for RNA High completely before use. Load your denatured RNA sample and 6 μl of DynaMarker® Prestain Marker for RNA High (use a 4~6 mm comb) on to a well and run the gel using 1 × MOPS electrophoresis buffer at 4~5 V / cm.

3) Transfer the DynaMarker® Prestain Marker for RNA High and RNA from the gel to positively charged nylon membranes. (see figure 3 and 4)

3-1) Place the gel in RNase-free glass dish, and rinse (for formaldehyde removal) with several changes of sufficient deionized water to cover the gel.
3-2) Add ~10 gel volumes of 3 M NaCl / 10 mM NaOH (transfer buffer) to the dish and soak for 30 min.
3-3) Cut a piece of nylon membrane slightly larger than the gel. Soak the membrane and two sheets of blotting paper of appropriate size in 10× SSC for at least 5 min.
3-4) Place the support (e.g. oblong sponge) in a glass or a plastic dish. Fill the dish with enough transfer buffer (soak the support about half-submerged in buffer.).
3-5) Place the gel on the support in inverted position so that it is centered on the wet blotting paper.
3-6) Place the wet nylon membrane on top of the gel. (**!** notice: Remove air bubbles.)
3-7) Place the wet blotting paper on the top of the wet nylon membrane. (**!** notice: Remove air bubbles.)
3-8) Cut a stack of paper towels (5~8 cm high), and place the towels on the blotting papers.
3-9) Put a glass plate on the top of the stack and weight it down with an about 400 g weight.
3-10) Allow upward transfer of RNA to occur for 1hr.
3-11) Transfer the membrane to a glass tray containing 6× SSC, and rinse for 5 min.
3-12) Remove the membrane from the 6× SSC and allow excess fluid to drain away. Then dry the membrane on blotting paper for a few minutes.
3-13) Fix the RNA to the membrane with a UV-crosslinker.
3-14) Cut off the marker lane.
3-15) Carry out northern hybridization.

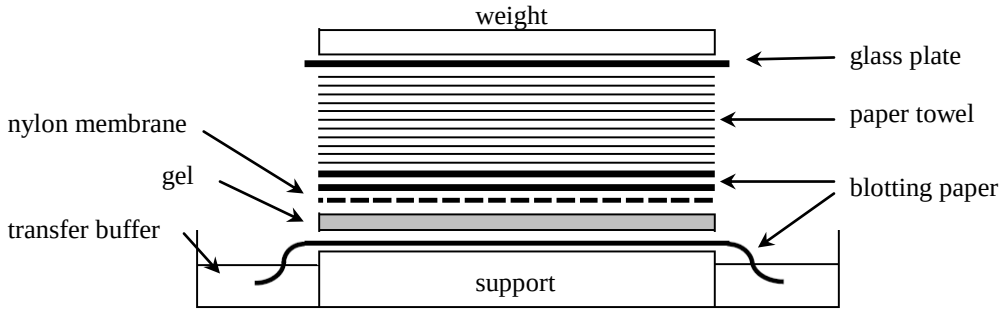


Figure 3: Upward Capillary Transfer.

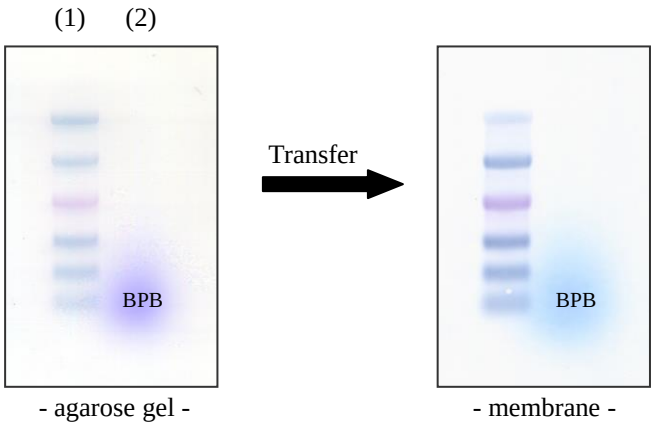


Figure 4: Left : Electrophoresis profile of DynaMarker® Prestain Marker for RNA High (1) and RNA sample (2) on 0.8 % agarose – 2.2 M formaldehyde gel / 1 × MOPS buffer as running buffer.
Right : Blotting of (1) and (2) onto nylon membrane.

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.