

日本語データシート

<u>Product Name</u>: DynaMarker® Prestain Marker for Small RNA Plus

Code No.: DM253

Range: 20 - 100 bases

Size: 150 μl (30 loadings)

Storage: store at -20 °C

#### **Description:**

The <sup>DynaMarker®</sup> 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 <sup>DynaMarker®</sup> Prestain Marker for Small RNA Plus is suitable for monitoring denaturing polyacrylamide gel electrophoresis and blotting onto membranes. The apparent sizes of bands in <sup>DynaMarker®</sup> 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 <sup>DynaMarker®</sup> 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.

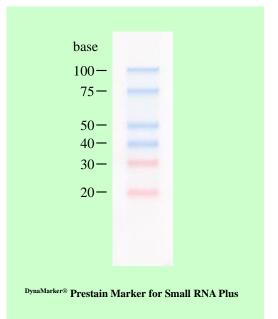


Figure 1. Electrophoresis profile of DynaMarker®

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  $^{\circ}$ 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 ul		

#### Note:

- For accurate electrophoretic determination of molecular weights, the <sup>DynaMarker®</sup> Small RNA II (code # DM192) or <sup>DynaMarker®</sup> Small RNA II Easy Load (code # DM197) should be used.
- A migration of the <sup>DynaMarker®</sup> 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.

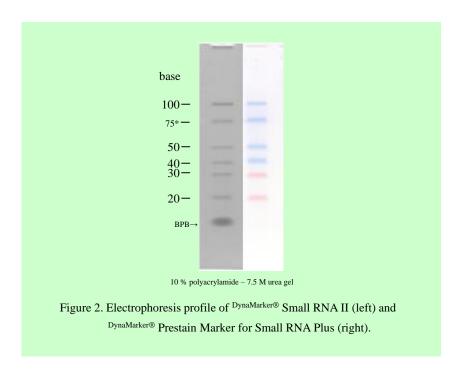


					acrylar	nide concentrat	tion: acrylamide:bis = 29:1, $1 \times TBE$ )		
			5.0 %	7.5 %	10 %	12.5 %	15 %	17.5 %	20 %
II	100 ba	ase	105.6 %	105.6	101.6	98.4	97.2	93.6	92.6
RNAII	75*		106.2	104.7	103.5	99.5	98.5	94.7	92.4
1RN	50		101.4	101.4	101.1	98.7	97.5	95.0	92.2
Small			103.1	102.0	103.2	100.8	100.0	97.4	93.9
	30		91.0	96.9	98.2	98.9	99.2	99.5	98.8
DynaMarker	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.)



## **Recommended usage:**

The <sup>DynaMarker®</sup> 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
$10 \times \text{TBE}$	2.0 ml
H2O	to 20 ml

After urea is dissolved completely, add 20  $\mu$ l of TEMED and 160  $\mu$ 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  $\mu l$  of  $^{DynaMarker\$}$  Prestain Marker for Small RNA Plus into a well and run the gel using  $1\times TBE$  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  $2 \times SSC$ .
  - 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.

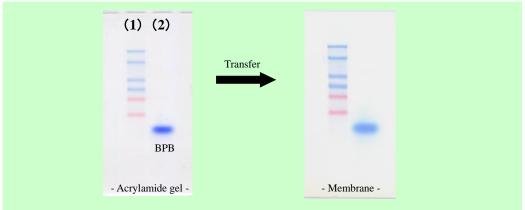


Figure 3. Left : Electrophoresis profile of (1)  $^{DynaMarker@}$  Prestain Marker for Small RNA Plus and (2) RNA sample on 10 % polyacrylamide - 7.5 M urea gel / 1  $\times$  TBE buffer as running buffer.

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.

# **Related Products:**

DM260	DynaMarker® Prestain Marker for RNA High Prestained marker for RNA(200-8000 bases)
DM192	DynaMarker® Small RNA II RNA marker (20-100 bases)
DM152	DynaMarker® RNA Low II RNA marker (20-500 bases)
DM170	DynaMarker® RNA High for Easy Electrophoresis RNA marker (200-8,000 bases) & RNA Loading Buffer. RNA sample can be electrophoresed on non-denaturing agarose gel as well as on denaturing agarose gel with this Loading Buffer.
DM660	DynaMarker® Protein MultiColor Stable II Prestained protein marker. Stable at 4°C.