

Product Name: DynaMarker Small RNA II Easy Load

Code No.: DM197

Range: 20-100 bases of RNA **Size:** 25 lodings, 125 µl

Loading: 5 μl is recommended for loading to a well

This product is research use only

Description:

The ^{DynaMarker} Small RNA II Easy Load has five single-stranded RNAs, 20, 30, 40, 50 and 100 bases, which is useful for a research of small RNAs. The 20, 30, 40 and 50 bases RNA are synthesized by chemically (non phosphorylated). The 100 bases RNA is synthesized by *in vitro* transcription. The ^{DynaMarker} Small RNA II Easy Load is supplied in a ready-to-use mixture of loading dye and RNAs (containing formamide, EDTA sodium salt, bromphenol blue). It is manufactured for denaturing polyacrylamide gel electrophoresis. The ^{DynaMarker®} Small RNA II Easy Load can be visualized by UV light exposure after ethidium bromide staining

Storage condition:

Store at -80 °C.

Repeated freeze/thaw cycles should be avoided.

Quality Control:

After 18 hrs incubation of the DynaMarker Small RNA II Easy Load at 37 °C, no visible degradation of the marker is observed in 12.5 % polyacrylamide / 7.5 M urea gel electrophoresis.

base - 100 - 50 - 40 - 30 - 20

DynaMarker Small RNA II
Easy Load

Electrophoresis profile of **DynaMarker** Small RNA II Easy Load (5 μl) on 12.5 % of acrylamide, 7.5 M urea gel with

1 × TBE buffer as running buffer

Supplied product: RNA Loading buffer PA

RNA Loading buffer PA is manufactured for denaturing polyacrylamide gel electrophoresis. The loading buffer has a composition of 80 % formamide, 10 mM EDTA sodium salt (pH 8.0), 0.025 % bromphenol blue. Store RNA loading buffer PA at -80 $^{\circ}$ C. Repeated freeze/thaw cycles should be avoided. It is 1 × to 2 × solution. Use RNA Loading buffer PA more than one volume of RNA solution.

Note

- The DynaMarker Small RNA II Easy Load is not prepared for estimating of RNA amount.
- The Small RNA II Easy Load should be run on 10-20% denaturing polyaceylamide gel for sizing RNAs.
- RNA is very sensitive to degradation by nucleases. To avoid damaging the ^{DynaMarker} Small RNA II Easy Load, use extreme care during manipulations to prevent nuclease contamination. Wear gloves and use clean apparatus. Glassware should be pretreated with diethyl pyrocarbonate (DEPC). Nuclease-free disposable plasticware should be used. Solutions and reagents to mix the marker should be high grade and nuclease-free. To use, thaw the ^{DynaMarker} Small RNA II Easy Load on ice and keep it on ice while using. For heat denaturation, transfer aliquot of the ^{DynaMarker} Small RNA II Easy Load to another tube, then heat it. Avoid repeated heat denaturizing.
 - ‡ Formamide is suspected to be harmful. It is irritate to the eyes and skin. Wear appropriate gloves and safety glasses. Put a lid tightly at the time of storage



Recommended usage:

• The ^{DynaMarker} Small RNA II Easy Load is suitable for 10-20% denaturing polyacrylamide gel electrophoresis. As recommended usage, ^{DynaMarker} Small RNA II Easy Load can be run on 12.5 % polyacrylsmide / 7.5 M urea gel as below.

- · Procedure
- 1. Preparation of 40 % Acrylamide : bis solution

After mixing, filter the solution through a nitrocellulose filter (0.45 µm pore size).

2. Preparation of 12.5 % polyacrylamide / 7.5 M urea gel (20 ml gel)

 $\begin{array}{lll} 40 \ \% \ acrylamide : bis \ solution & 6.25 \ ml \\ Urea & 9.0 \ g \\ 10 \times TBE & 2.0 \ ml \\ H_2O & to \ 20 \ ml \end{array}$

After urea is dissolved completely, add 20 μ l of TEMED and 160 μ l of 10 % ammonium persulfate. Mix quickly and then pour the gel into the mold of a vertical gel apparatus (8.7 cm \times 6.8 cm, thickness 1 mm). The gel apparatus should be assembled according to the manufacture's protocol and ready to run with 1 \times TBE buffer.

3. Loading and electrophoresis

Mix RNA to be analyzed (for example, RNA transcript) and RNA Loading buffer PA as below.

RNA sample dried precipitate or 2 μ l (0.5-2 μ l) RNA Loading buffer PA 5 μ l --- over one volume of RNA sample

Mix in a small tube, total 5-7 μl

Transfer aliquot (5-10 μ l) of DynaMarker Small RNA II Easy Load to a small tube. Heat RNA mixed with loading buffer PA, and DynaMarker Small RNA II Easy Load at 80°C for 5 min, and immediately transfer the tube on ice. Load the mixture onto a well of 12.5 % polyacrylamide / 7.5 M urea gel and start electrophoresis. After the tracking dyes have migrated an appropriate distance through gel, stop the electrophoresis. To stain with ethidium bromide, disassemble the apparatus and transfer the polyacrylamide gel to a gel tray filled with 1 \times TBE buffer containing 1.0 μ g/ml ethidium bromide. Stained RNA can be visualized using UV transilluminator.

Reference:

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Related products

	DM253	DynaMarker Prestain Marker for Small RNA Plus
		Pre-stained molecular weight marker for a small size RNA. (range: 20~100 bases)