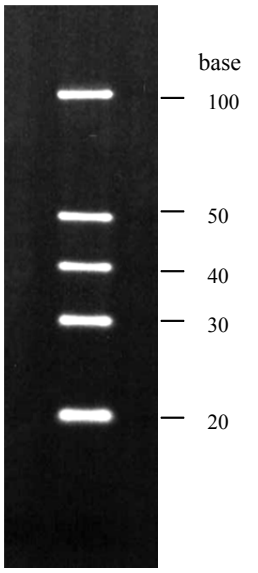
PRODUCT INFORMATION

Product Name : DynaMarker Small RNA Ⅱ Easy Load
Code No. : DM197
Range : 20-100 base of RNA
Size : 25 lodings, 125 µl
Loading : 5 µl is recommended for loading to a well

This product is research use only

Description :

Small RNAs, miRNA and siRNA, have drawn much attention recently. 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 are synthesized by chemically (non phosphorylated). The 100 bases 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 or by staining with Gel Indicator™ RNA Staining Solution (DM590, 595)



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

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.

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℃. Repeated freeze/thaw cycles should be avoided. It is 1 × to 2 × solution. Use 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

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Recommended usage :

・The DynaMarker Small RNA Ⅱ Easy Load is manufactured 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

Acrylamide : bis	190 g
N, N-methylenebisacrylamide	10 g
ddH_2O	to 500 ml

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)

40 % acrylamide : bis solution	6.25 ml
Urea	9.0 g
10 × TBE	2.0 ml
H_2O	to 20 ml

After urea is dissolved completely, add 20 μl of TEMED and 160 μl of 10 % ammonium persulfate. Mix quickly and then pour the gel into the mold of a vertical gel apparatus (8.7 cm × 6.8 cm, thickness 1 mm). The gel apparatus should be assembled according to the manufacture's protocol and ready to run with 1 × 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 μl (0.5-2 μl)
RNA Loading buffer PA	5 μl --- over one volume of RNA sample
	Mix in a small tube, total 5-7 μl

Transfer aliquot (5-10 μl) 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℃ 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 × TBE buffer containing 1.0 μg/ml ethidium bromide. Stained RNA can be visualized using UV transilluminator.

Alternatively, Gel Indicator™ RNA staining solution is used for the gel staining. After electrophoresis, transfer polyacrylamide gel to a tray containing distilled water to rinse the gel for a few minutes. Discard water in the tray. And add Gel Indicator™ RNA staining solution (x 1) so that acrylamide gel is covered. Stain the gel for 20-30 minutes with gentle agitation. RNA will be visible as a blue band.

Reference :

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