

PRODUCT INFORMATION

Product Name : DynaMarker Small RNA II
Code No. : DM192
Range : 20-100 base of RNA
Size : 30 loadings, 30 μ l (approximately 6-8 μ g)

This product is research use only

Description :

Small RNAs include a variety of non-coding RNAs, such as miRNA, siRNA, snoRNA and snRNA. Recently such a small RNA is intensively studied, because the small RNA has been found to control many biological events. The DynaMarker Small RNA II consists of five single-stranded RNAs (ssRNA). The 20, 30, 40 and 50 bases RNAs are synthesized by chemically (non phosphorylated). The 100 bases RNA is synthesized by *in vitro* transcription. The DynaMarker Small RNA II is suitable for determining size of small-size ssRNA in denaturing polyacrylamide gel electrophoresis. The DynaMarker Small RNA II can be visualized by ethidium bromide staining or by staining with Gel Indicator™ RNA Staining Solution (DM590, 595).

Storage buffer :

10 mM Tris-HCl (pH 8.0) buffer containing 1 mM EDTA
(Ammonium acetate is slightly contained)

Storage condition :

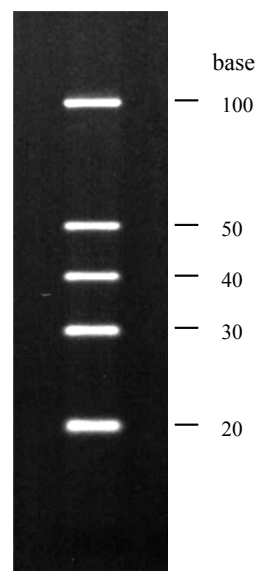
Store at -80 °C. Repeated freeze/thaw cycles should be avoided.

Quality Control :

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

Note :

- The DynaMarker Small RNA II is not prepared for estimating of RNA amount.
- The DynaMarker Small RNA II should be run on 10-20% denaturing polyacrylamide gel for sizing RNAs.
- RNA is very sensitive to degradation by nucleases. To avoid damaging the DynaMarker Small RNA II, 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 on ice and keep it on ice while using.



DynaMarker Small RNA II

Electrophoresis profile of
DynaMarker Small RNA II (1 μ l) on
12.5 % of acrylamide, 7.5 M urea gel with
1 \times TBE buffer as running buffer

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Recommended usage :

- The ^{DynaMarker} Small RNA II is manufactured for 10-20% denaturing polyacrylamide gel electrophoresis. As recommended usage, ^{DynaMarker} Small RNA II can be run on 12.5 % polyacrylamide / 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 ₂ O | 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 ₂ O | 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 manufacturer's protocol and ready to run with 1 × TBE buffer.

3. Loading and electrophoresis

Mix 5 µl of gel loading buffer* with 1 µl** of ^{DynaMarker} Small RNA II or a few µg of RNA sample in a small tube. Heat 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 × TBE buffer containing 1.0 µg/ml ethidium bromide. Stained RNA can be visualized using UV transilluminator.

gel loading buffer*

| | |
|--------------|---------------------|
| 80 % | deionized formamide |
| 0.025% (w/v) | bromophenol blue |
| 0.025% (w/v) | xylene cyanol FF |
| 10 mM | EDTA (pH8.0) |

** The amount is enough to be visualized by ethidium bromide staining.

Reference:

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