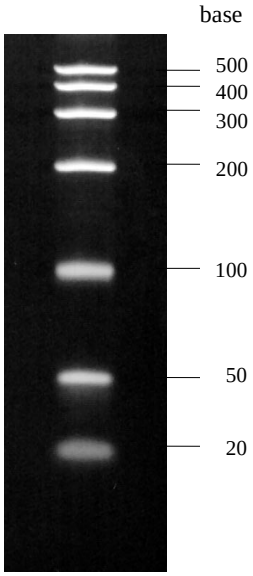
PRODUCT INFORMATION

Product Name : DynaMarker RNA Low II
Code No. : DM152
Range : 20-500 base of RNA
Size : 50 μg (72 μl), 0.7 mg/ml

This product is research use only

Description :

The DynaMarker RNA Low II consists of seven single-stranded RNAs. The 20-base and 50-base RNA are synthesized by chemically (not phosphorylated). The 100, 200, 300, 400 and 500 bases are synthesized by *in vitro* transcription. The DynaMarker RNA Low II is suitable for determinating size of single-stranded RNAs in denaturing polyacrylamide gel electrophoresis. The concentration of each RNA (20-500 base) in the marker is approximately 0.1 μg/μl. It is useful for estimating of RNA amount. The DynaMarker RNA Low II can be visualized by ethidium bromide staining or by Gel Indicator™ RNA Staining Solution (DM590, 595).



DynaMarker RNA Low II
Electrophoresis profile of DynaMarker RNA Low II (0.7 μg) on 5 % of acrylamide, 8 M urea gel with 1 × TBE buffer as running buffer

Storage buffer :

10 mM Tris-HCl (pH 8.0) buffer containing 1 mM EDTA

Storage condition :

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

Quality Control : After 18 hr incubation of the DynaMarker RNA Low II at 37 °C, no visible degradation of the marker is observed in 5 % polyacrylamide / 8M urea gel electrophoresis

Note :

RNA is very sensitive to degradation by nucleases. To avoid damaging the DynaMarker RNA Low 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 RNA Low II on ice and keep it on ice while using.

Recommended usage :

The DynaMarker RNA Low II is suitable for RNA size determining in denaturing polyacrylamide gel electrophoresis. For one of example, DynaMarker RNA Low II can be run on 5 % polyacrylamide / 8M urea gel as below. Effective range of separation of RNAs is about 50-500 base in 5 % polyacrylamide / 8M urea gel.

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Procedure

1. Preparation of 40 % Acrylamide :bis solution

Acrylamide	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 5 % polyacrylamide / 8M urea gel (20 ml gel)

40 % acrylamide : bis solution	2.5 ml
Urea	9.6 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 (7 cm × 8 cm, thickness 1.0 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 5 μl of gel loading buffer* with 1 μl (0.7 μg) ** of ^{DynaMarker} RNA Low II or a few μg of RNA sample in a small tube. Heat at 80 ^{o}C for 3 min and immediately transfer the tube on ice. Load the mixture onto a well of 5 % polyacrylamide / 8M 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 10 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.

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