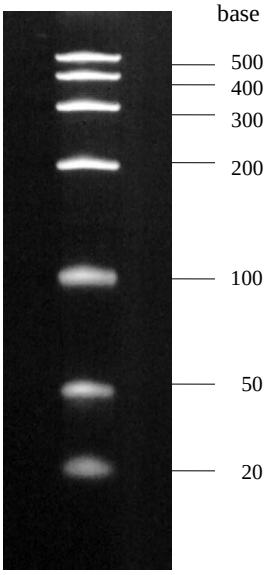
Product Name : DynaMarker RNA Low II Easy Load
Code No. : DM157
Range : 20-500 base of RNA
Size : 125 µl, about 25 loadings
Loading : 5 µl is recommended for loading to a well
(0.1 µg of each RNA / 5 µl)

This product is research use only

Description :

The DynaMarker RNA Low II Easy Load is supplied in a ready-to-use mixture of loading dye (containing formamide, EDTA sodium salt, bromphenol blue) and RNAs. It is prepared for denaturing polyacrylamide gel electrophoresis but not agarose gel elctrophoresis. The DynaMarker RNA Low II Easy Load has seven single-stranded RNAs, 20, 50, 100, 200, 300, 400 and 500 bases. The 20-base and 50-base RNA are synthesized by chemically (not phosphorylated), others are synthesized by *in vitro* transcription. In 5 µl of the DynaMarker RNA Low II, each RNA amount is approximately 100 ng. It is useful for estimating RNA amount approximately. The DynaMarker RNA Low II Easy Load can be visualized by UV light after ethidium bromide staining.



DynaMarker RNA Low II Easy Load

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

Storage condition :

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

Quality Control :

After 18 hr incubation of the DynaMarker RNA Low II Easy Load at 37 ºC, no visible degradation of the marker is observed in 5 % polyacrylamide / 8M urea gel electrophoresis.

Supplied product : RNA Loading buffer PA

RNA Loading buffer PA is manufactured for denaturing polyacrylamide gel electrophoresis but not agarose gel elctrophoresis. The loading buffer has a composition of 80 % formamide, 10 mM EDTA sodium salt (pH8.0), 0.025 % bromphenol blue. Store RNA Loading buffer PA at -80 ºC. Repeated freeze/thaw cycles should be avoided. It is 1 × to 2 × solution. Use more than one volume of RNA solution.

Note :

RNA is very sensitive to degradation by nucleases. To avoid damaging the DynaMarker RNA Low 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 product should be high grade and nuclease-free. To use, thaw the DynaMarker RNA Low II Easy Load on ice and keep it on ice while using. For heat denaturation, transfer aliquot of the DynaMarker RNA Low 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.

BioDynamics Laboratory Inc.
www.biodynamics.co.jp

Recommended usage :

The DynaMarker RNA Low II Easy Load is manufactured for denaturing polyacrylamide gel electrophoresis. As recommended usage, DynaMarker RNA Low II Easy Load is 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.

Procedure

1. Preparation of 40 % Acrylamide : bis solution

Acrylamide	190 g
N, N-methylenebisacrylamide	10 g
H_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 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 μg)
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 RNA Low II Easy Load to a small tube. Heat RNA mixed with RNA Loading buffer PA and DynaMarker RNA Low II Easy Load at 80 ºC for 3 min, and transfer the tube on ice immediately, then load onto a well of 5 % polyacrylamide / 8M urea gel and start electrophoresis. After the tracking dye has 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.

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:

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.