PRODUCT INFORMATION

Product Name : DynaMarker RNA High  
Code No. : DM160  
Range : 200-8,000 base of RNA  
Size : 50 μg (56 μl), 0.9 mg/ml  

This product is research use only

Description :
The DynaMarker RNA High consists of nine single-stranded RNAs, 200, 500, 1,000, 1,500, 2,000, 3,000, 4,000, 5,000 and 8,000 bases, which are synthesized by in vitro transcription. The DynaMarker RNA High is suitable for determining size of single-stranded RNAs in denaturing agarose gel electrophoresis. The concentration of each RNA (200-8,000 base) in the marker is approximately 0.1 μg/μl. It is useful for estimating of RNA amount. The DynaMarker RNA High can be visualized by UV light after ethidium bromide staining or exposure to film with end labeling.

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 High at 37 °C, no visible degradation of the marker is observed in formaldehyde-agarose (1%) gel electrophoresis.

Note :  
RNA is very sensitive to degradation by nucleases. To avoid damaging the DynaMarker RNA High, 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 High on ice and keep it on ice while using.

Recommended usage :  
The DynaMarker RNA High is suitable for RNA size determining in denaturing agarose gel electrophoresis. For one of example, DynaMarker RNA High can be run on denaturing agarose gel containing formaldehyde below.

Procedure  
1. Agarose gel containing formaldehyde  
Add 1 g of agarose to 85 ml of H2O in a flask, dissolve the agarose in a microwave. Add 10 ml of 10 × MOPS buffer to the agarose solution, then allow it in a flask to cool to 55 °C. Add 5.4 ml of 37 % formaldehyde solution to the agarose solution, mix them, quickly pour the agarose into a gel mold and set a comb in a fume hood. Cover the gel with 1 × MOPS buffer until use.
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Formaldehyde is supplied as a 37-40 % W/V (12.3 M) solution that contain a stabilizer such as methanol (10-15 %). The 37 % formaldehyde solution is used for denaturing agarose gel containing formaldehyde. For instance, Sigma-Aldrich supplies formaldehyde solution, 36.5-38 % in water, for molecular biology, which contains 10-15 % methanol.

10 × MOPS buffer *  0.2 M  MOPS
20 mM  Sodium acetate
10 mM  EDTA (pH 8.0)

2. Denaturation of RNA
Prepare denaturated DynaMarker RNA High and RNA to be analysed in a small tube as below.

DynaMarker RNA High or RNA*  2 µl
10 × MOPS buffer  2 µl
37 % formaldehyde solution  4 µl
formamide  10 µl
200 µg/ml ethidum bromide  1 µl

After mixing, heat the RNA solution at 75 °C for 3 min, then quickly transfer the tube on ice.

* Required RNA amount depends on experiments. For northern analysis, up to 15 µg of RNA is loaded. For detection of DynaMarker RNA High by ethidium bromide staining, load 0.5-4 µg of the marker.

3. Loading and electrophoresis
Add 2 µl of 10 × formaldehyde gel-loading buffer* to each RNA solution and return the tube on ice.

Set up the prepared agarose gel containing formaldehyde in a horizontal electrophoresis apparatus submerged in 1 × MOPS buffer. Load the denatured RNA solution to a well and start electrophoresis. After the tracking dyes have migrated an appropriate distance through gel, stop the electrophoresis. RNA bands can be seen under UV illumination.

10 × formaldehyde gel-loading buffer*  50 %  glycerol
10 mM  EDTA (pH 8.0)
0.025 % (w/v)  bromophenol blue
0.025 % (w/v)  xylene cyanol FF

Reference: