

PRODUCT INFORMATION

日本語データシート



Product Name : DynaMarker dsRNA Easy Load
Code No. : DM185
Range : 10 - 1,000 base of RNA
Size : 125 μ l, about 25 loadings
Loading : 5 μ l is recommended for loading to a well

This product is research use only

Description :

The DynaMarker dsRNA Easy Load is supplied in a ready-to-load mixture of loading dye (containing Tris-HCl buffer, glycerol, EDTA sodium salt, sodium chloride, bromphenol blue) and an ideal size marker for determining sizes of double-stranded RNAs. The DynaMarker dsRNA Easy Load consists of ten double-stranded RNAs, 10, 20, 30, 50, 100, 200, 300, 400, 500 and 1,000 base pairs. In 5 μ l of the DynaMarker dsRNA Easy Load, a 20 bp of dsRNA is approximately 50 ng. The DynaMarker dsRNA Easy Load can be visualized by UV light after ethidium bromide staining.

Storage condition :

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

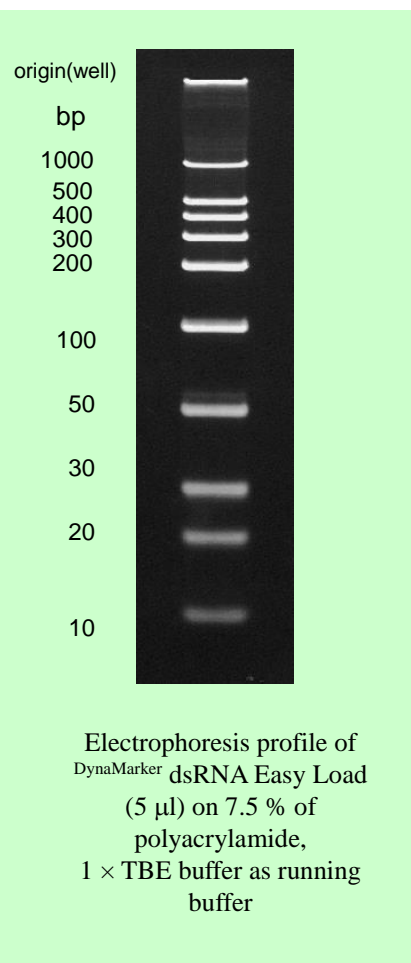
Quality Control : After 18 hr incubation of the DynaMarker dsRNA Easy Load at 37°C, no visible degradation of the marker is observed in 7.5 % polyacrylamide gel electrophoresis

Supplied product : 6 × dsRNA Loading Buffer

6 × dsRNA Loading Buffer is used for preparation of dsRNA samples for non-denaturing polyacrylamide gel electrophoresis. One volume of 6 × dsRNA Loading Buffer is added to 5 volumes of sample. The 6 × dsRNA Loading Buffer is RNase free and contains Tris-HCl buffer (pH7.5), glycerol, EDTA sodium salt, bromphenol blue. Store at - 20 °C or - 80 °C.

Note :

Even dsRNA is more resistant to RNase than ssRNA, dsRNA is sensitive to degradation by RNase. To avoid damaging the DynaMarker dsRNA, use 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 dsRNA Easy Load on ice and keep it on ice while using.



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Recommended usage :

The ^{DynaMarker} dsRNA Easy Load is manufactured for non-denaturing polyacrylamide gel electrophoresis. As recommended usage, ^{DynaMarker} RNA Easy Load is run on 7.5 % polyacrylamide gel as below.

Procedure

1. Preparation of 7.5 % polyacrylamide gel (20 ml gel)

40 % acrylamide : bis solution (29:1)	3.75 ml
10 × TBE	2.0 ml
H ₂ O	to 20 ml

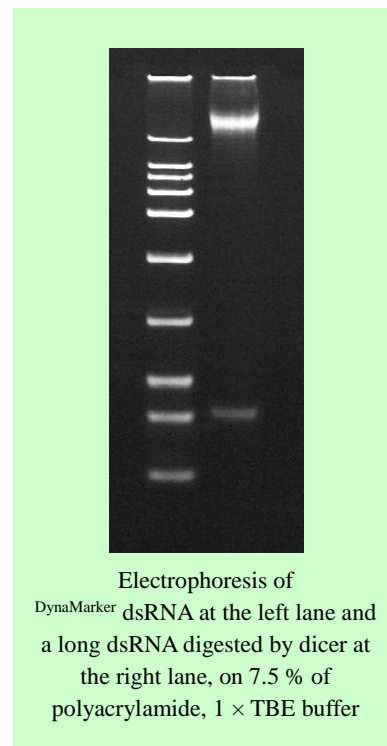
2. After mixing reagents described above, add 20 µl of TEMED and 160 µl of 10 % ammonium persulfate. Mix quickly and then pour the gel into the mold of a vertical gel apparatus (20 ml is enough gel solution for two 7 cm × 8 cm, thickness 0.1 cm gels). The gel apparatus should be assembled according to the manufacture's protocol and ready to run with 1 × TBE buffer.

3. Loading and electrophoresis

Prepare dsRNA sample for electrophoresis as below.

1) Size Marker:		
^{DynaMarker} dsRNA Easy Load		5 µl
2) Sample to examine:		
dsRNA sample		X µl (*)
Nuclease-free water		4 – X µl
<u>6 × dsRNA Loading Buffer</u>		<u>1 µl</u>
		Total 5 µl

(*) 50-500 ng of dsRNAs.



Electrophoresis of ^{DynaMarker} dsRNA at the left lane and a long dsRNA digested by dicer at the right lane, on 7.5 % of polyacrylamide, 1 × TBE buffer

Mix dsRNA solution with 6 × dsRNA Loading Buffer in a tube as above. Load the mixture onto a well of 7.5 % polyacrylamide 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.

Reference:

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