Product Name : DynaMarker RNA High for Easy Electrophoresis
(Previous name : DynaMarker RNA Easy Measurement N)

Code No. : DM170 *This product is research use only*

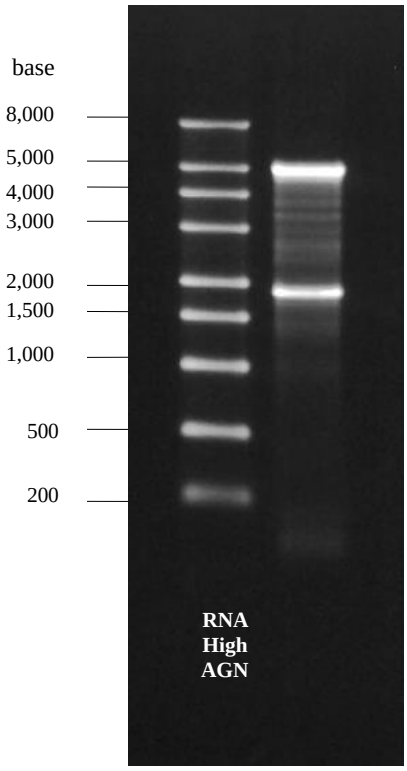
Components:

Code No.	Component	Content	Amount
DM171	DynaMarker RNA High AGN Store at -80 °C	RNAs prepared by in vitro transcription Range: 200-8,000 bases of RNA	25 µg, 0.9mg/ml about 25 loadings
DM172	RNA loading buffer AG+ Store at -80 °C	3-N-morpholino propansulfonic acid, sodium acetate, ethidium bromide, glycerol, formamide, EDTA sodium salt, bromphenol blue.	1 ml

† Formaldehyde is not included in this Product.

Description :

The DynaMarker RNA High for Easy Electrophoresis is manufactured for easy measurement of RNA size on agarose gel electrophoresis. With DynaMarker RNA High for Easy Electrophoresis, electrophorsis is possible on non-denaturing agarose gel as well as on denaturing agarose gel. To use DynaMarker RNA High for Easy Electrophoresis, just mix RNA loading buffer AG with formaldehyde solution (not included in this kit), and added the mixture to DynaMarker RNA High AGN or RNA sample, then heat to load into wells of agarose gel. The RNA loading buffer AG+ is a gel loading buffer prepared not only for denaturing agarose gel electrophoresis but also for non-contained denaturing agarose gel electrophoresis. DynaMarker RNA High AGN contains nine single-stranded RNAs, 200, 500, 1,000, 1,500, 2,000, 3,000, 4,000, 5,000 and 8,000 bases. Each RNA is 0.1 µg/µl approximately. It is useful for estimating RNA amount approximately. After electrophoresis, RNA bands on agarose gel can be visualized by UV light without further ethidium bromide staining.



Electrophoresis profile of DynaMarker RNA High AGN on formaldehyde-agarose (1%) gel.
Left lane : 0.45 µg of DynaMarker RNA High AGN
Right lane : 0.4 µg of Human Total RNA

Storage condition :

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

Quality control :

DynaMarker RNA High AGN :

After 18 hr incubation of DynaMarker RNA High AGN at 37 °C, no visible degradation of the marker is observed in formaldehyde-agarose (1%) gel electrophoresis.

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www.biodynamics.co.jp

Note :

RNA is very sensitive to degradation by nuclease. To prevent nuclease contamination, extreme care is required for manipulation. 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 AGN on ice. Keep DynaMarker RNA High AGN and RNA loading buffer AG+ on ice while using. This kit can be used for non-denaturing agarose gel electrophoresis. Although adequate results to size RNAs were obtained as far as we tested, denaturing agarose gel electrophoresis may be required for a strict experiment.

‡ Formamide is suspected to be harmful. It is irritate to the eyes and skin. Ethidium bromide is a strong mutagen and susupected to be toxic. Wear appropriate gloves and safety glasses in using solutions and materials containing formamide and/or etidium bromide. Put a lid tightly at the time of storage.

‡ Formaldehyde is carcinogen and toxic. Avoid breathing the vapors. Wear appropriate gloves and safety glasses. Gel containing formaldehyde should be prepared and used in a chemical hood.

Recommended usage :

Using DynaMarker RNA High for Easy Electrophoresis, RNA electrophorsis is possible on non-denaturing agarose gel (1 × TAE, 0.5 × TBE) as well as on denaturing agarose gel. To size RNAs very strictly, denaturing agarose gel electrophoresis may be required.

< Procedure for non-denaturing agarose gel electrophoresis >

1. Preparation of non-denaturing agarose gel

Add 1.3 g of agarose to 100 ml of 1 × TAE in a flask, dissolve the agaose in a microwave. After swirling to mix them, quickly pour the agarose into a gel mold and set a comb just as agarose gel for DNA electrophoresis.

2. Formaldehyde –added RNA loading buffer AG+ is prepared as below.

RNA loading buffer AG+	95 µl
37 % formaldehyde solution	5 µl
Formaldehyde –added RNA loading buffer AG+*	100 µl

* After mixing with formaldehyde, the solution is not stable, do not use it more than 6 hours after preparation.

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 mixing with RNA loading buffer AG+ and also 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.

3. Denaturation of RNA

Prepare denaturated DynaMarker RNA High AGN and RNA sample to be analysed in a small tube as below.

DynaMarker RNA HighAGN or RNA sample	0.5-2 µl *
formaldehyde–added RNA loading buffer AG+**	3 µl***
ddH_2O	to 5 µl
	5 µl ¶

After mixing, heat the RNA solution at 75 ºC 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 HighAGN by UV illumination (ethidum bromide staining), load 0.5-4 µl of the marker. More than 0.05 µg of RNA band can be detected on gel under UV light with treatment of formaldehyde-added RNA loading buffer AG+.
** Use freshly prepared formaldehyde-added RNA loading buffer AG+.
*** It is important to add constant volume of formaldehyde-added RNA loading buffer AG+ to all RNA solutions which are electrophoresed in gel, especially for estimation of RNA amount. The formaldehyde-added RNA loading buffer AG+ is 1 × to 2 × buffer. Use more than one volume of RNA solution.
¶ Mixture of formaldehyde-added RNA loading buffer AG+ and RNA is not stable, use it promptly.

4. Loading and electrophoresis
Set up the prepared agarose gel in a horizontal electrophoresis apparatus submerged in 1 × TAE. Load the above denatured RNA solution* to a well and start electrophoresis as just DNA electrophoresis. After the tracking dye has migrated an appropriate distance through gel, stop the electrophoresis. RNA bands can be seen under UV illumination.

< Procedure for denaturing agarose gel electrophoresis >

1. Preparation of denaturing agarose gel
Add 1 g of agarose to 85 ml of H_2O in a flask, dissolve the agaose 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. 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 mixing with RNA loading buffer AG+.

2. Formaldehyde-added RNA loading buffer AG+ is prepared as below.

RNA loading buffer AG+	95 µl
37 % formaldehyde solution	5 µl
Formaldehyde-added RNA loading buffer AG+*	100 µl

* After mixing with formaldehyde, the solution is not stable, do not use it more than 6 hours after preparation.

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

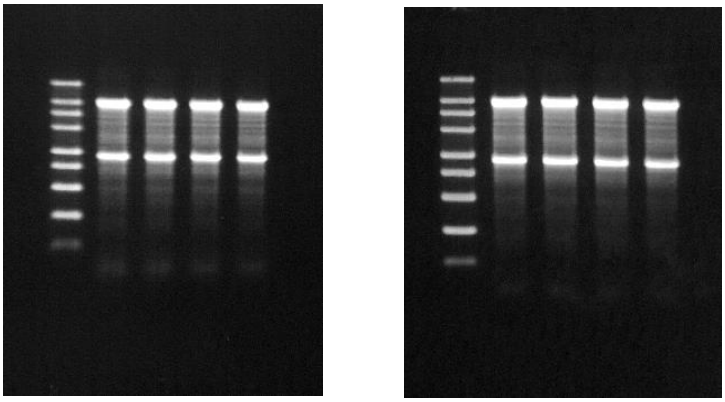
DynaMarker RNA HighAGN or RNA sample	0.5-2 µl *
formaldehyde–added RNA loading buffer AG+**	3 µl***
ddH_2O	to 5 µl
	5 µl ¶

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 HighAGN by UV illumination (ethidum bromide staining), load 0.5-4 µl of the marker. More than 0.05 µg of RNA band can be detected on gel under UV light with treatment of formaldehyde-added RNA loading buffer AG+.

** Use freshly prepared formaldehyde-added RNA loading buffer AG+.
*** It is important to add constant volume of formaldehyde–added RNA loading buffer AG+ to all RNA solutions, which electrophoresed in gel, especially for estimation of RNA amount. The formaldehyde –added RNA loading buffer AG+ is 1 × to 2 × buffer. Use more than one volume of RNA solution.
¶ Mixture of formaldehyde-added RNA loading buffer AG+ and RNA is not stable, use it promptly.

4. Loading and electrophoresis
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 dye has migrated an appropriate distance through gel, stop the electrophoresis. RNA bands can be seen under UV illumination.

Profile of electrophoresis



Denaturing agarose gel Non-denaturing agarose gel.

DynaMarker RNA High AGN (0.45 μg/ well), Human Total RNA (0.4 μg/ well) were electrophoresed on denaturing agarose gel (left) or non-denaturing agarose gel (right). DynaMarker RNA High AGN or Human Total RNA was mixed with formaldehyde-added RNA loading buffer AG+ and treated as above.

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