Product name : RNA Ezloading Dye (2X)
(Previous product name: RNA loading buffer AG+)
Product Code : DM 172
Size: 1ml

This product is for research use only.

Composition: Formamide, Ethidium Bromide, Bromophenol blue, Buffer solution
The product does not contain formaldehyde.

Description :
This product enables RNA electrophoresis on non-denaturing agarose gels as well as denaturing agarose gels, and RNA bands can be visualized using a UV transilluminator after electrophoresis without the need for ethidium bromide staining.
To use, simply add formaldehyde to the RNA Ezloading Dye (formaldehyde is not included in this kit), mix it with the RNA sample, and heat it.

Storage conditions :
−20 °C or below

Note :
RNA is highly sensitive to degradation by nucleases. To prevent nuclease contamination, please take utmost care when handling the sample. Wear laboratory gloves and use clean equipment. Treat glassware with DEPC beforehand. Alternatively, we recommend using nuclease-free disposable plasticware.

Use high-grade, nuclease-free reagents for preparing RNA samples and markers.

Keep this product on ice during use.

This product is prepared for electrophoresis using non-denaturing agarose gels, but for rigorous experiments, please also consider using denaturing agarose gel electrophoresis.

Formamide is known to be harmful to the eyes and skin. Ethidium bromide is a mutagen and is suspected to be toxic. Wear appropriate protective equipment when working with solutions containing formamide or ethidium bromide. Store solutions with the lid tightly closed.

Formaldehyde is a carcinogen. Do not breathe vapors and use appropriate protective equipment when handling. Handle gels containing formaldehyde in a local exhaust hood.

Recommended Use :

This product allows electrophoresis in both denaturing agarose gels and non-denaturing agarose gels (TAE or TBE buffer). However, to precisely determine the RNA size, electrophoresis in a denaturing agarose gel may be necessary.

< Non-denaturing agarose gel electrophoresis >

1. Preparation of non-denaturing agarose gel

Add 100 ml of 1x TAE buffer and 1.3 g of agarose to a flask, melt it in a microwave, stir well, and then quickly pour it into an agarose gel tray and set the comb.

2. Prepare formaldehyde-added RNA Ezloading Dye.

Example:	RNA Ezloading Dye	19 µl
	37% formaldehyde solution	1 µl
	Formaldehyde-added RNA Ezloading Dye *	20 µl

* Ezloading Dye is unstable after adding formaldehyde, so please use it within 6 hours after preparation. Use 37% formaldehyde for RNA Ezloading Dye preparation.

3. RNA denaturation

Mix an equal or lesser volume of sample with formaldehyde-added RNA loading dye.

Example:	RNA sample	X µl *
	formaldehyde-added RNA Ezloading Dye**	2.5 µl***
	ddH2O	to 5 µl
		5 µl**

After mixing, heat at 75° C for 3 minutes and immediately place the tube on ice.

* The amount of RNA required varies depending on the experiment. Formaldehyde-added RNA Ezloading Dye treatment allows detection of 0.05 µg of RNA band in the gel under UV illumination.

** The mixture of formaldehyde-added RNA Ezloading Dye and RNA sample is unstable. Please use it promptly.

***It is important to add the same volume of Formaldehyde-added RNA Ezload Dye to all RNA samples to be electrophoresed, especially when estimating the amount of RNA.

4. Loading and Electrophoresis

The prepared agarose gel is placed in a submarine electrophoresis apparatus filled with 1x TAE buffer. As with DNA electrophoresis, the denatured RNA solution is loaded into the wells and electrophoresis is immediately started. When the dye reaches the appropriate position on the gel, electrophoresis is stopped, and the gel is observed on a transilluminator.

< Denaturing agarose gel electrophoresis >

1. Preparation of denaturing agarose gel

Place 85 ml of purified water and 1 g of agarose in a flask and dissolve the agarose in a microwave. Next, add 10 ml of 10x MESA buffer. Once the solution in the flask has cooled to 55℃, add 5.4 ml of 37% formaldehyde* solution in a fume hood and mix well. Then quickly pour the mixture into an agarose gel preparation plate and insert the comb.

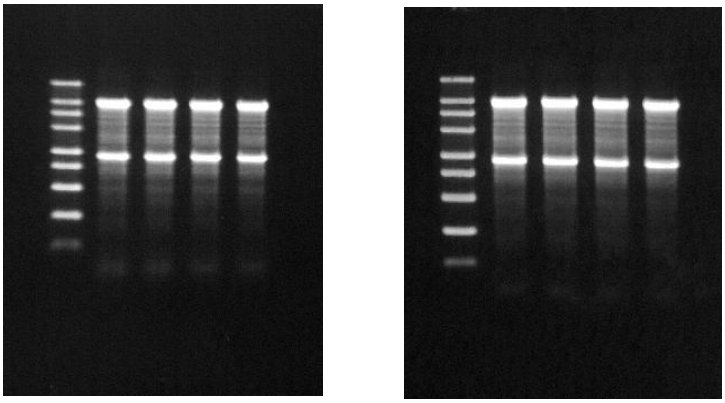
Once the gel has set, cover with 1x MESA buffer until ready to use.
*Concentrated formaldehyde is 37–40% W/V (12.3M).

2. Preparation of formaldehyde-added RNA Ezloading Dye (perform the same procedure as above for <Non-denaturing agarose gel electrophoresis>)

3. RNA denaturation (performed in the same manner as in <Non-denaturing agarose gel electrophoresis> above)

4. Loading and Electrophoresis
The prepared agarose gel is placed in a submarine electrophoresis apparatus filled with 1x MESA buffer. The denatured RNA solution is loaded into the wells and electrophoresis is immediately started. Electrophoresis is stopped when the dye reaches the appropriate position on the gel. The RNA bands are observed under a UV transilluminator.

Electrophoresis



Denaturing agarose gel　　Non-denaturing agarose gel

RNA was run similarly on denaturing and non-denaturing gels.

DynaMarker® RNA High or Human Total RNA was treated with formaldehyde-containing RNA Ezloading Dye.

Reference:

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

Related Products

DM160	DynaMarker® RNA High RNA molecular weight markers ranging from 200 to 8000 bases