

PRODUCT INFORMATION

Product Name : DynaExpress miRNA fractionation Kit AV

Code No. : DS315AV

This product is research use only

Kit Components: 10 preparations

Components	Amount	Storage
RNA Extraction Buffer	15 ml	- 20 °C
Colored Guide Size Marker	200 µl	- 20 °C
RNA Loading buffer PA	1 ml	- 20 °C
Glycogen (5 µg/µl)	260 µl	- 20 °C
Sodium Acetate Solution (3 M)	1.3 ml	- 20 °C

- RNA Extraction Buffer : 20 mM Tris-HCl, (pH 8), 0.1 mM EDTA, 0.3 M NaCl, 0.1 % SDS
- RNA Loading buffer PA contains 90 % formamide. Formamide is suspected to be harmful. It is an irritant to the eyes and skin. Wear appropriate gloves and safety glasses. Put a lid tightly at the time of storage.

Introduction:

Non coding small RNAs, such as miRNA, siRNA, have been revealed to be important molecules in many kinds of biological events. It is an efficient way and a practically cost-effective method to purify small RNAs by using denaturing-polyacrylamide gel electrophoresis. To fractionate miRNAs using this DynaExpress miRNA fractionation Kit, total RNA is electrophoresed on denaturing polyacrylamide gel and miRNAs of 18-28 nucleotides are excised and extracted from polyacrylamide gel on the base of mobility of Colored Guide Size Marker.

Feature and Specification :

- Cost-effective method to purify miRNA.
- No stain is required.
- Easy excision by indication of Colored Guide Size Marker.

Storage condition : Stable at -20 °C for 12 months from the date of receipt.

Related Products:

DS330	DynaExpress miRNA Cloning Kit High Efficient
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Protocol of miRNA fractionation:

Starting materials

Roughly speaking, one ng of miRNA is possibly recovered per 10 µg of total RNA. Total RNA can be prepared by guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski P. and Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction Anal. Biochem. 162:156-159.) or by commercial total RNA preparation kits. When using a commercial kit, be sure that the kit does not deplete small RNAs. Semi-purified miRNA prepared by using commercial kits which enrich small RNA less than five or two hundred bases can be also used for the miRNA fractionation Kit.

Colored Guide Size Marker

The Colored Guide Size Marker is designed for the purpose of fractionating small RNAs. It consists of six pre-stained oligo nucleic acids, I, II, III, IV, V, VI. (See Fig.1). The apparent size of band I and II correspond to that of 5'-phosphorylated 18 and 28 mer RNAs, respectively on denaturing polyacrylamide electrophoresis. The region corresponded to the size of miRNA. The apparent size of band III and IV correspond to that of MI-A3' Linker* ligated products (36, 46 mer) on denaturing polyacrylamide electrophoresis. Likewise, band V and VI correspond to MI-A3' Linker and MI-R5' Linker* ligated products (53, 63 mer).

MI-A3' Linker* and MI-R5' Linker* are components of ^{DynaExpress} miRNA Cloning Kit High Efficient (DS330).

Additional Materials Needed

- Micropipet
- Sterile 1.5 ml microfuge tubes
- Vortex mixer
- Chloroform
- Apparatus for polyacrylamide gel electrophoresis
- Power supply
- Polyacrylamide gel (15 %)
- 1 × TBE (Tris/Borate/EDTA buffer)
- Shaker or agitator
- Micropipet tips
- Microcentrifuge
- TE-saturated phenol
- Ethanol (absolute)
- Bunsen burner
- Clean razor blade

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Experiment

1. Preparation of polyacrylamide gel

1) Preparation of 40 % acrylamide : bis solution

Acrylamide	190 g
N, N'-methylenebisacrylamide	10 g
H ₂ O	to 500 ml

After mixing, filter the solution through a membrane filter (0.45 µm pore size).

2) Preparation of 15 % polyacrylamide /7.5 M urea gel

Prepare urea/acrylamide solution as follows:

40 % acrylamide : bis solution	7.5 ml
Urea	9.0 g
10 × TBE	2.0 ml
H ₂ O	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, 1.5 mm thickness, lane width = 12 mm). Let the gel sit until it is solid. The gel apparatus should be assembled according to the manufacturer's protocol and ready to run with 1 × TBE buffer.

2. Electrophoresis of total RNA

Total RNA, 100-250 µg, is ethanol precipitated and dissolved with a small amount of nuclease-free water. It is important to load a volume of RNA solution equal or nearly equal to that of Colored Guide Size Marker. Volume must be adjusted as indicated in Table 1. Colored Guide Size Marker, 20 µl, may be also mixed with 10 µl of RNA Loading buffer PA.

Table 1 Loading of total RNA for gel electrophoresis

	RNA	Colored Guide Size Marker
Total RNA* (µg)	100-250	20 µl
Nuclease-Free Water (µl)	10-15	0 µl
RNA Loading buffer PA (µl)	10-15	0-10 µl
Total volume (µl)	20-30	20-30 µl
Lane(s)* to load	1 lane	1 lane

Total RNA*: ethanol precipitate

Lane(s)*: 1.5 mm thickness, 12 mm width. One load should be 30 µl or less than 30 µl

RNA should be completely dissolved by gentle mixing. If RNA solution is not completely dissolved, the volume may increase slightly by adding of RNA Loading buffer PA. In this case, adjust the loading volume of Colored Guide Size Marker solution to that of RNA solution. It is important to load an equal or nearly equal volume of solution to a lane to ensure similar gel running behavior. Heat the prepared RNA solution for 3 min at 70 °C and transfer on ice immediately (Heat treatment is not necessary for Colored Guide Size Marker). Rinse the wells of the gel with 1 × TBE and make sure the lanes are flat for good loading and resolution. Load the RNA in a lane. Load the volume-adjusted Colored Guide Size Marker

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solution to another lane next to the lane of the total RNA. Electrophorese the gel with a constant voltage of about 150-200V, until the Bromophenol blue dye reaches a point of 2-3 cm from the bottom.

3. Extraction of miRNA

After polyacrylamide gel electrophoresis, dismantle the gel-running apparatus. Detach only one of two glass plates from gel. (Now, the gel is on the other glass plate.) Transfer the gel on the glass plate to the surface of a clean white tray. Compare Colored Guide Size Marker and sample RNA, and cut out the gel in the lane of sample RNA corresponding to band I to II of Colored Guide Size Marker (see Fig. 1). Band I and band II of Colored Guide Size Marker correspond to 18 and 28 nt RNA, respectively. Use a clean razor blade for excision. Transfer the excised gel to a nuclease-free 1.5-ml tube and weight the gel. Heat a clean 200 μ l-pipette tip in a flame quickly to make round its end and cool it down. Use the pipette tip as a pestle and grind the gel completely. Add 3 volumes of RNA Extraction Buffer. Elute the RNA from the

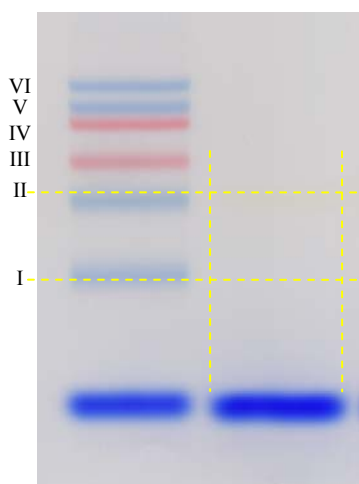


Fig. 1 Excision of 18 – 28 mer small RNAs.

Colored Guide Size Marker (left lane, 20 μ l) and Total RNA from HL60 cells (100 μ g/lane) prepared as described in text and were electrophoresed on 15 % polyacrylamide gel (7.5 M urea, 1 \times TBE). The rectangles surrounded by broken yellow lines represent portions to be excised.

gel by constant agitation at room temperature for 30 min. Centrifuge the tube at top speed (15,000 rpm) for 10 min using a microcentrifuge in the temperature range from 4°C to room temperature to precipitate gel pieces. Transfer the supernatant to a nuclease-free 1.5-ml tube. Add an equal volume of TE-saturated phenol to the supernatant. Vortex it for 15-30 sec. Centrifuge the tube at top speed for 5 min using a microcentrifuge at 4°C to room temperature. Transfer the supernatant to another nuclease-free 1.5-ml tube. Add an equal volume of chloroform to the supernatant. Vortex it briefly. Centrifuge the tube at top speed for 2 min at 4°C to room temperature. Transfer the supernatant to another nuclease-free 1.5-ml tube. Perform chloroform extraction as above again. Add 1/10 volume of 3 M NaOAc solution and 1/50 volume of 5 μ g/ μ l of glycogen. Mix it and centrifuge briefly to collect the contents at the bottom of the tube, then add three volumes of 100 % ethanol to the solution. Put the tube at - 80°C for over 30 min, then centrifuge the tube at top speed for 10 min using a microcentrifuge at 4°C. To remove supernatant, centrifuge the tube again at top speed for 2 min at 4°C. Remove the supernatant completely and dry down the precipitate. The recovered precipitate is a small RNA fraction of 18 - 28 mer. Store it at - 80°C until use.