NAUTIAGENE

Product Information

NautiaZ microRNA Mini Kit

(50 prep)

Cat.No. : NGMRZ-S050

Sample : 10^7 Culture cells
300 µl blood
10^9 Bacteria cells
10^8 Fungi cells

Yield : Up to 30 µg

NGCRZ-S100	NautiaZ Cell/Blood Total RNA Mini Kit (100 prep)
NGCRZ-S300	NautiaZ Cell/Blood Total RNA Mini Kit (300 prep)
NGBRZ-S100	NautiaZ Bacteria/Fungi RNA Mini Kit (100 prep)
NGBRZ-S300	NautiaZ Bacteria/Fungi RNA Mini Kit (300 prep)
NGTRZ-S100	NautiaZ Tissue Total RNA Mini Kit (100 prep)
NGTRZ-S300	NautiaZ Tissue Total RNA Mini Kit (300 prep)
NGPRZ-S100	NautiaZ Plant Total RNA Mini Kit (100 prep)
NGPRZ-S300	NautiaZ Plant Total RNA Mini Kit (300 prep)
NGMRZ-S050	NautiaZ microRNA Mini Kit (50 prep)
NGVN-S100	NautiaZ Viral Nucleic Acid Extraction Kit (100 prep)
NGVN-S300	NautiaZ Viral Nucleic Acid Extraction Kit (300 prep)

Contents

	NGMRZ-S050T	NGMRZ-S050
ML Buffer	2 ml x2	60 ml
MO Buffer	1 ml	15 ml
MA Buffer	2 ml	25 ml
W1 Buffer	2 ml	25 ml
W2 Buffer*	300 ul x2	15 ml
Elution Buffer	1 ml	10 ml
Red Column	4 pcs	50 pcs
Blue Column	4 pcs	50 pcs
Collection Tube	8 pcs	100 pcs
User Manual	1	1

*Add 1.2 ml x2 / 60 ml ethanol (96-100%) to W2 Buffer prior to the initial use

Buffer Preparation

• Add ethanol (96-100%) to the Wash Solution prior to first use:

	NGMRZ-S050T	NGMRZ-S050
W2 Buffer ethanol (96 ~ 100%)	300 ul x2 1.2 ml x2	15 ml 60 ml

Optional Step (DNA Residue Degradation)

Add 5 µl of 10X Reaction Buffer and 5 µl of DNase I, Amplification Grade, and incubating to the **50 µl final product.** Let stand for 15 minutesat room temperature. Inactivate the DNase by **adding 5 µl of the Stop Solution** and heating at 70 °C for 10 minutes. (Suggesting product: Sigma #AMPD1-1KT, DNase I, Amplification Grade)

Description

NautiaZ microRNA Mini Kit provides a fast, simple, and cost-effective method for isolation of microRNA from whole blood, mammalian cells and bacterial cells. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system further allows microRNA bases to bind to the the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and pure microRNA is eluted with Elution Buffer without phenol extraction or alcohol precipitation needs. microRNA purified with NautiaZ microRNA Mini Kit is suitable for a variety of routine applications including gene regulation and functional The entire procedure can be completed within 25-40 minutes.

Important Notes

1. Buffer contains chaotropic salt is harmful and irritant agent.
2. Use sterile, RNase-free pipet tips and microcentrifuge tube. Wear a lab coat and disposable gloves to prevent RNase contamination.
3. Make sure the starting sample amount is under the limit.
4. Add ethanol (96-100%) to W2 Buffer prior to the initial use.
5. All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g (~14000 x g).

Additional Requirements

1. ß-Mercaptoethanol
2. RNase-free microcentrifuge tubes
3. 70% ethanol

For Bacteria sample:

1. Lysozyme Buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)

For Fungus sample:

1. Lyticase or Zymolase
2. Sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1M Tris-Cl pH 7.5; 35 mM mercaptoethanol)

For Culture Cell

STEP	PROCEDURE
1 Sample prepare	Transfer up to 10^7 Culture mammalian cells to a micracentrifuge tube *(not provided).* Centrifuge at 6,000 x g for 1 min and remove the supernatant completly.
	Add 100 µl ML Buffer to the tube and resuspend the cell pellet by vortexing or pipetting.

For Blood

STEP	PROCEDURE
1 Sample prepare	Collect blood in EDTA-Na2 treated collection tubes. Transfer up to 300 µl blood to a 1.5 ml tube. **Add 900 µl ML Buffer,** then mix by insertion. Incubate the mixture on ice for 10 minutes, and invert every 5 minutes.
	Centrifuge at 4,000 x g for 5 minutes at 4°C. Remove the supernatant completely. **Add 100 µl ML Buffer** and resuspend the pellet by pipetting.

For Gram+ Bacteria

STEP	PROCEDURE
1 Sample prepare	Transfer up to 10^9 bacteria cells to a micracentrifuge tube *(not provided).* Centrifuge at 12,000 x g for 1 min and remove the supernatant completly.
	Add 200 µl Lysozyme Buffer (20mg/ml lysozyme) to the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 10 minutes.

For Gram- Bacteria

STEP	PROCEDURE
1 Sample prepare	Transfer up to 10^9 bacteria cells to a micracentrifuge tube *(not provided).* Centrifuge at 12,000 x g for 1 min and remove the supernatant completly.
	Add 100 µl MO Buffer to the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 5 minutes.

For Fungus

STEP	PROCEDURE
1 Sample prepare	**Transfer up to 10^8 fungus cells** to a microcentrifuge tube. Centrifuge at 6,000 x g for 5 mins. Remove the supernatant completely. Resuspend the cell with **600 µl Sorbitol Buffer** by pipetting. **Add 200 U of lyticase or zymolase**. Incubate at 30°C for 30 minutes.
	Centrifuge the mixture at 2,000 x g for 10 mins. Remove the supernatant completely.
	Add 100 µl MO Buffer to the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 5 minutes.

PURIFICATION PROTOCOLS

STEP	PROCEDURE
2 Cell Lysis	**Add 300 µl MA Buffer** and **3 µl ß-Mercaptoethanol**, mix by vortexing. Incubate at room temperature for 5 minutes.
	Centrifuge at 14,000 x g for 10 minutes. Transfer the supernatant to a clean microcentrifuge tube.
3 Protein remove	**Add 300 µl 70% ethanol** and mix by vortexing. Place a **Red column** in Collection Tube. Transfer the sample mixture (up to 700 µl once) to Red column and centrifuge 1 minute at 14,000 x g. Transfer the the flow-through to a new 1.5 ml microcentrifuge tube. **Add 600 µl 70% ethanol** and mix by vortexing. Place a **Blue column** in Collection Tube.
4 RNA Binding	Transfer the sample mixture (up to 700 µl once) to Blue column and centrifuge 1 minute at 14,000 x g. Discard the flow-through and place Blue Column back in the Collection tube.
5-1 Wash	**Add 400 µl W1 Buffer** to Blue Column. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place **Blue Column** back in the Collection tube.
5-2 Wash	**Add 600 µl W2 Buffer** (ethanol added) to **RZ Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place **RZ Column** back in the Collection tube.
6 Dry	Centrifuge at 14,000 x g for 2 minutes to dry the column.
7 Elution	Place **Blue Column** to a RNase-free 1.5 ml tube. **Add 50 µl of preheated Elution Buffer** (75°C) into the center of the column matrix.
	Stand at room temperature for 2 minutes. Centrifuge at 14,000 x g for 2 minutes to elute RNA.
8 Pure RNA	Store the RNA fragment at -80 °C.