NAUTIAGENE

Product Information

NautiaZ Bacteria/Fungi RNA Mini Kit

(100/300 prep)

Cat.No. : NGBRZ-S100/NGBRZ-S300

Sample : 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	Nautia Viral Nucleic Acid Extraction Kit (100 prep)
NGVN-S300	Nautia Viral Nucleic Acid Extraction Kit (300 prep)

Ver. 2020-01

Contents

	NGBRZ-S100T	NGBRZ-S100	NGBRZ-S300
BRO Buffer	1 ml	25 ml	65 ml
BRA Buffer	2 ml	45 ml	125 ml
W1 Buffer	2 ml	45 ml	125 ml
W2 Buffer*	300 ul x2	15 ml	25 ml x2
Elution Buffer	1 ml	10 ml	30 ml
RZ Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
User Manual	1	1	1

*Add 1.2 ml x2 / 60 ml / 100 ml x2 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:

	NGBRZ-S100T	NGBRZ-S100	NGBRZ-S300
W2 Buffer ethanol (96 ~ 100%)	300 ul x2 1.2 ml x2	15 ml 60 ml	25 ml x2 100 ml x2

Additional Requirements

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

For Bacteria sample:

4. 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:

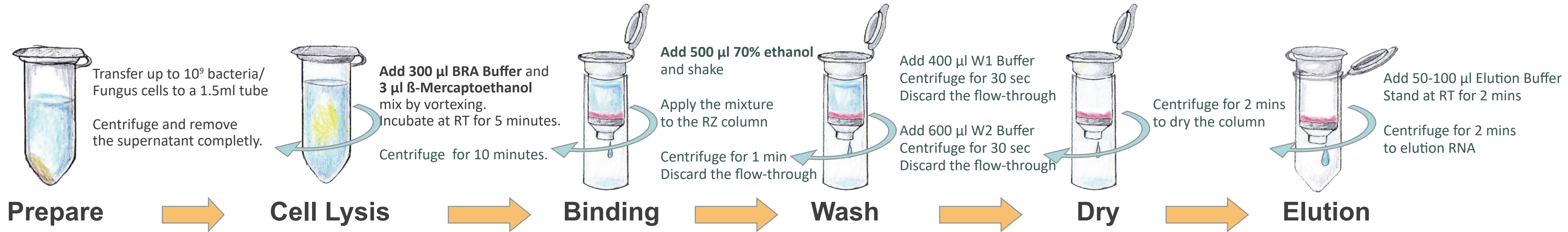
4. Lyticase or Zymolase
5. Sorbitol buffer (1.2 M sorbitol; 10 mM CaCl2; 0.1M Tris-Cl pH 7.5; 35 mM mercaptoethanol)

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).

For Fungus

STEP	PROCEDURE
1 Sample prepare	**Transfer up to 10^8 fungus cells** to a microcentrifuge tube *(not provided).* 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 200 µl BRO Buffer to the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 5 minutes.
	Add 300 µl BRA Buffer and **3 µl ß-Mercaptoethanol**, mix by vortexing. Incubate at room temperature for 5 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 complety.
	Add 200 µl BRO Buffer to the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 5 minutes.
	Add 300 µl BRA Buffer and **3 µl ß-Mercaptoethanol**, mix by vortexing. Incubate at room temperature for 5 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 complety.
	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.
	Add 300 µl BRA Buffer and **3 µl ß-Mercaptoethanol**, mix by vortexing. Incubate at room temperature for 5 minutes.

PURIFICATION PROTOCOLS

STEP	PROCEDURE
2 Cell Lysis	Centrifuge at 14,000 x g for 10 minutes. Transfer the supernatant to a clean microcentrifuge tube.
3 RNA Binding	**Add 500 µl 70% ethanol** and shake vigorously. Place a **RZ column** in Collection Tube. Transfer the sample mixture (up to 700 µl once) to RZ column and centrifuge 1 minute at 14,000 x g. Discard the flow-through and place RZ Column back in the Collection tube.
4-1 Wash	**Add 400 µl W1 Buffer** 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.
Optional Step: DNase	*If DNA-free RNA is required, perform this optional step.* **Add 150 µl W2 Buffer (ethanol added)** into the RZ column. Centrifuge at full speed (16,000 x g) for 30 seconds. Discard the flow-through and place the RZ Column back in the Collection Tube.
	For each isolation reaction , premix **80 µl DNase I Incubation Buffer** with **2 µl DNase I** in a new sterile tube (Do not vortex!). **Add 82 µl of the DNase I solution** into the center of the RZ Column membrane and incubate at room temperature for 15 min.
4-2 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.
5 Dry	Centrifuge at 14,000 x g for 2 minutes to dry the column.
6 Elution	Place **RZ Column** to a clean 1.5 ml microcentrifuge tube *(not provided)*. **Add 50-100 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 purified RNA.
7 Pure RNA	Store the RNA fragment at -80 °C.