

# NAUTIAGENE

## Product Information

## NautiaZ Cell/Blood Total RNA Mini Kit

(100/300 prep)

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

Sample :  $10^7$  Culture cells

300  $\mu$ l Blood

Yield : Up to 30  $\mu$ g

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. 2020-06

## Contents

	NGCRZ-S100T	NGCRZ-S100	NGCRZ-S300
CR1 Buffer	2 ml x2	110 ml	110 ml x3
CR2 Buffer	2 ml	45 ml	125 ml
W1 Buffer	2 ml	45 ml	125 ml
W2 Buffer*	300 $\mu$ l 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:

	NGCRZ-S100T	NGCRZ-S100	NGCRZ-S300
W2 Buffer ethanol (96 ~ 100%)	300 $\mu$ l x2 1.2 ml x2	15 ml 60 ml	25 ml x2 100 ml x2

## Additional Requirements

1. RNase-free microcentrifuge tubes
2. 70% ethanol
3.  $\beta$ -Mercaptoethanol

## Important Notes

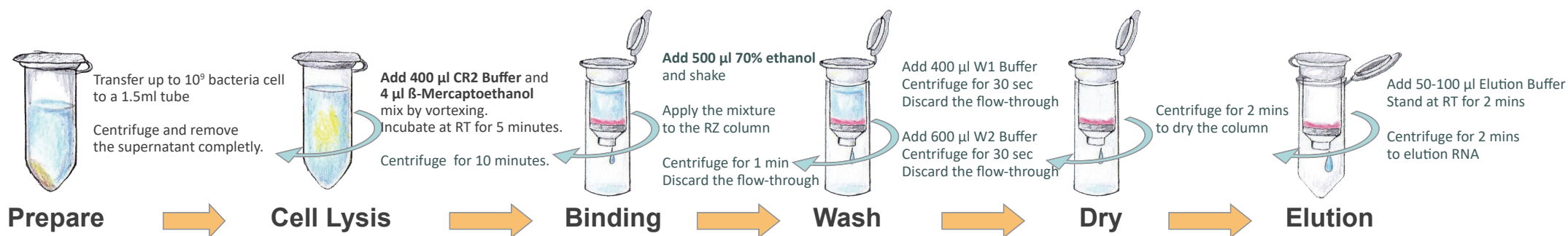
1. Buffer contains chaotropic salt is harmful and irritant agent.
2. Use sterile, RNase-free pipet tips and microcentrifuge tube. **Wear a lab coat and disposable gloves to prevent RNase contamination.**
3. Make sure the starting sample amount is under the limit.
4. Add ethanol (96-100%) to W2 Buffer prior to the initial use.
6. All purification steps should be carried out at room temperature.
7. All centrifugations should be carried out in a table-top microcentrifuge at  $>12000 \times g$  (10,000-14,000 rpm, depending on the rotor type).

## Quality Control

The quality of NautiaZ Cell/Blood RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 300  $\mu$ l of fresh human blood or  $10^7$  Culture cells. More than 1  $\mu$ g of total RNA was quantified with a spectrophotometer and checked by formaldehyde agarose gel analysis. Finally, RT-PCR was used to ensure the quality of total RNA.

## Storage

Store at room temperature.



## For Culture Cells

STEP	PROCEDURE
1 Sample prepare	Transfer up to $10^7$ mammalian cells to a microcentrifuge tube ( <i>not provided</i> ). Centrifuge at 6,000 x g for 1 min and remove the supernatant completely.
	<b>Add 100 <math>\mu</math>l CR1 Buffer</b> to the tube and resuspend the cell pellet by vortexing or pipetting.
	<b>Add 400 <math>\mu</math>l CR2 Buffer</b> and <b>4 <math>\mu</math>l <math>\beta</math>-Mercaptoethanol</b> , mix by vortexing. Incubate at room temperature for 5 minutes.

## For Blood

STEP	PROCEDURE
1 Sample prepare	Transfer up to 300 $\mu$ l blood to a RNase-free microcentrifuge tube ( <i>not provided</i> ). <b>Add 900 <math>\mu</math>l CR1 Buffer</b> , then mix by insertion. Incubate the mixture on ice for 10 minutes, and invert every 5 minutes.
	Centrifuge at 4°C at 4,000 x g for 5 minutes. Remove the supernatant completely. <b>Add 100 <math>\mu</math>l CR1 Buffer</b> and resuspend the pellet by pipetting.
	<b>Add 400 <math>\mu</math>l CR2 Buffer</b> and <b>4 <math>\mu</math>l <math>\beta</math>-Mercaptoethanol</b> , 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	<b>Add 500 <math>\mu</math>l 70% ethanol</b> and shake vigorously. Place a <b>RZ column</b> in Collection Tube. Transfer the sample mixture (up to 700 $\mu$ l 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	<b>Add 400 <math>\mu</math>l W1 Buffer</b> 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	<i>If DNA-free RNA is required, perform this optional step.</i> <b>Add 150 <math>\mu</math>l W2 Buffer (ethanol added)</b> 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 <b>80 <math>\mu</math>l DNase I Incubation Buffer</b> with <b>2 <math>\mu</math>l DNase I</b> in a new sterile tube ( <b>Do not vortex!</b> ). <b>Add 82 <math>\mu</math>l of the DNase I solution</b> into the center of the RZ Column membrane and incubate at room temperature for 15 min.

4-2 Wash	<b>Add 600 <math>\mu</math>l W2 Buffer</b> (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 ( <i>not provided</i> ). <b>Add 50-100 <math>\mu</math>l of preheated Elution Buffer (75°C)</b> into the center of the column matrix.
7 Pure RNA	Stand at room temperature for 3 minutes. Centrifuge at 14,000 x g for 2 minutes to elute purified RNA.
	Store the RNA fragment at -80 °C.