NAUTIAGENE

Product Information

NautiaZ Tissue Total RNA Mini Kit

(100/300 prep)

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

Sample: 30 mg of Tissue

25 mg of Paraffin Embedded Tissue

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)

Contents

	NGTRZ-S100T	NGTRZ-S100	NGTRZ-S300
TR 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:

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

Additional Requirements

- 1. ß Mercaptoethanol
- 2. Xylene
- 3. RNase-free microcentrifuge tubes
- 4. 70% ethanol

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 purification steps should be carried out at room temperature.
- 6. All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g (10,000-14,000 rpm, depending on the rotor type).

Quality Control

The quality of NautiaZ Tissue Total RNA Mini Kit is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from animal tissue. More than 1 μ g of total RNA was quantified with a spectrophotometer and checked by formaldhyde agarose gel analysis. Finally, RT-PCR was used to ensure the quality of total RNA.

Storage

Store at room temperature.





Cut off animal sample Grind the sample under liquid nitrogen to a fine

Add 400 ul TR Buffer Add 4 μl β-mercaptoethanol Incubate at 80 °C for 20 mins until it is completely dissolved.

Transfer sample mixture to new 1.5 ml tube Add 400 µl 70% ethanol Shake or pipetting.

Apply the mixture to the RZ column

Centrifuge for 1 min Discard the flow-through Add 400 µl W1 Buffer Centrifuge for 30 sec Discard the flow-through

Add 600 µl W2 Buffer Centrifuge for 30 sec Discard the flow-through

Centrifuge for 2 mins to dry the column

Add 50-100 µl Elution Buffer Stand at RT for 2 mins

Centrifuge for 2 mins to elution RNA

Prepare



Cell Lysis













Elution

For each isolation reaction, premix 80 ul DNase I

For Fresh Tissue

STEP	PROCEDURE
1 Sample prepare	Cut off 30 mg of fresh animal tissue and grind the sample using micropestle in a microcenterifuge tube. If sample is frozen, grind the sample under liquid nitrogen to a fine powder with pestle and mortar.

For Paraffin embedded Tissue

STEP	PROCEDURE
1 Sample prepare	Slice up to 25 mg of paraffin-embedded tissue and transfer to a 1.5 ml tube. Add 1 ml xylene and vortex vigorously and incubate at room temperature for 10 minutes. Vortex every 2 minutes during incubation.
	Centrifuge 3 minutes at 14,000 x g. Remove the supernatant. Add 1 ml absolute ethanol and mix by inverting.
	Centrifuge 3 minutes at $14,000 \times g$. Remove the supernatant. Add 1 ml absolute ethanol and mix by inverting.
	Centrifuge 3 minutes at $14,000 \times g$. Remove the supernatant. Open the tube and incubate at 37°C for 15 minutes.

PURIFICATION PROTOCOLS

STEP	PROCEDURE
2 Cell Lysis	Add 400 μ I TR Buffer and 4 μ I β -mercaptoethanoI to the sample. Grind the sample until the sample lysate is clear. Transfer the sample mixture to a RNase-free microcentrifuge tube and incubate at 80°C for 20 mins.
	Centrifuge 3 mins at $14,000 \times g$. Transfer the supernatant to a new 1.5 ml tube. Pre-heat the Elution Buffer at 75°C .
3 RNA Binding	Add 400 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 1 : 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.

	Step 2	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 μ l 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 3 minutes. Centrifuge at $14,000 \times g$ for 2 minutes to elute purified RNA.
	7 Pure RNA	Store the RNA fragment at -80 °C.