NOVA Tissue and Mammalian Cell Culture RNA extraction Kit (WF-1010400X)

Overview

The *NOVA* Tissue and Mammalian Cell Culture RNA extraction kit provides a time-efficient and simple method to extract total RNA from mammalian tissue samples with ease and precision. This product will allow the user to extract total RNA using silica-based membrane technology with an easy and quick to use spin column. The protocol has been designed to maximise RNA yield and purity, whilst minimising degradation and removing inhibitors, reducing problems in downstream applications.

Stages

1. Cell Lysis
2. RNA Binding
3. Washing
4. Elution

Features of *NOVA* Tissue and Mammalian Cell Culture RNA extraction kit

Efficient – Once cell lysis is complete, this 15-minute protocol allows easy transition straight into downstream applications.

High yield – the automation in the extraction procedure minimises product loss.

Versatile – the RNA product can be used in many downstream applications such as PCR, RT-qPCR, and RNA sequencing.

High Purity – our product allows you to exclude enzyme inhibitors, DNA and proteins from the samples in order to easily transition to your downstream applications.

Product information

Catalog. number	No. of reactions
WF10104001	50
WF10104002	250

Required materials

- Mammalian tissue or cells from culture
- Pipettes and tips
- Vortex
- Water bath or Hot plate
- Nuclease-free Eppendorf's
- 100% ethanol
- Centrifuge
- Timer

Reaction timings

The *NOVA* Tissue and Mammalian Cell Culture RNA extraction Kit will be complete in 15 minutes following cell lysis. The RNA product will be ready to use for a variety of downstream applications. Upon extraction, RNA should be stored at a maximum of -20^{0}C to avoid sample degradation. To avoid further degradation, we recommend aliquoting your RNA to reduce freeze thaw cycles.

Storage conditions

The reagents provided in the *NOVA* Tissue and Mammalian Cell Culture RNA extraction Kit should be stored at room temperature.

Warning!

RW2 buffer requires the addition of 100% ethanol, before extraction take place.

Please be advised to wear appropriate eyewear, clothing and gloves. Read the Safety Data Sheets (SDS) for any further safety information. When working with RNA, ensure RNase free working conditions.

Any further information

For further information and protocols, visit our website https://willowfort.co.uk/. For any additional support, please contact us through https://willowfort.co.uk/contact-us

For research use only. These products should not be used for diagnostics

Recommended Tissue and Cell Quantities

The table below suggests the weight or amount of tissue required for this products protocol:

Table 1: Tissue types, amounts and their expected RNA yield

Tissue type	Weight or amount	RNA yield
Mouse tail	0.5cm	10-20µg
Mammalian tissue	20-25mg	10-30µg
Cultured cells	$1x10^7$	10-30µg
Yeast cells	$2x10^8$	10-30µg

Components of *NOVA* Tissue and Mammalian Cell Culture RNA extraction Kit

TRH buffer 1 (30ml) – using salts and chelating agents, this reagent destroys the cell membrane and begins digesting proteins, lipids and carbohydrates.

RW1 buffer (30ml) – This buffer washes a large proportion of impurities from the RNA sample, increasing RNA purity,

RW2 buffer (25ml) – This reagent includes a mix of ethanol and water to remove salts from the RNA, increasing the purity of the final sample. ***Before extraction, please add 75ml of 100% ethanol****

Nuclease-free water (15mL)

NOVA Tissue and Mammalian Cell Culture RNA extraction Kit protocol

Stage 1: Lysis

1. Allow all reagents and samples to equilibrate at room temperature.
2. Add your sample in accordance to Table 1 to a nuclease-free Eppendorf. Add 400µl TRH buffer 1 and 4µl of β-mercaptoethanol to the sample.
3. Vortex the sample thoroughly and incubate at room temperature for 3 minutes.
4. Set up the filter column and add the lysis sample to the centre of the filter.
5. Centrifuge the sample at 1000g at room temperature for 30 seconds.
6. Discard the filter after all the lysis solution has been collected.

Stage 2: RNA binding

7. Set up the **RNA** spin column/tube before beginning this next stage.
8. To precipitate the RNA, add 400µl of 70% ethanol to the sample and mix thoroughly at room temperature.
9. Add the sample/ethanol solution to the **RNA** spin column and centrifuge at 14000g for 30-60 seconds. Discard the flow through.
10. Repeat step 8, until all the sample/ethanol solution has been centrifuged. *Optional: add DNase I solution (1:4) to the filter and spin.*

Stage 3: Washing

11. Add 400µl of RW1 buffer to the spin column and centrifuge at 14000g for 1 minute. Discard the flow through.
12. Add a further 600µl of RW2 buffer to the spin column and centrifuge again at 14000g for 1 minute. Discard the flow through.

Stage 4: Elution

11. Before elution, centrifuge the spin column for 2-3 minutes at 16000g without adding any solutions to the column
12. Remove the spin column from the original collection tube and replace with a fresh nuclease free Eppendorf.
13. Add 50µl nuclease-free water to the spin column directly in the middle of the filter. *Optional step: incubate the spin column with the nuclease-free water for up to 1 minute to increase yield.*
14. Elute the newly extracted RNA from the spin column by centrifuging for 14000g for 1 minute.
15. *Optional: To increase RNA integrity, add DNase I solution (1:4) to the filter and spin your extraction RNA sample.*
16. Store the newly extracted RNA in -20^0C until required for further experiments.

Additional protocol information

Sample volume requirement – Please see table 1 for sample amounts and the expected RNA yield.

Elution volume – Depending on your required final RNA concentration, please add 50µl of nuclease-free water.

Troubleshooting – For any troubleshooting queries, please visit our website http://willowfort.co.uk/sample-preparation/nova-tissue-and-mammalian-cell-culture-rna-extraction-kit. Any further information, please contact us, via https://willowfort.co.uk/contact-us