



GENETIX BRAND

HANDBOOK



Nucleopore[®] RNA Clean & Concentrator Kit

Nucleopore[®] RNA Clean & Concentrator Kit NP- 7101R 50 Preps



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COMPONENTS

Kit contents

Nucleopore® RNA Clean & Concentrator Kit

Cat #	NP-7101R
No. of Preps	(50 preps.)
RNA Binding Buffer (RCB)	25 ml
RNA Prep Buffer (RCP)	25 ml
RNA Wash Buffer1 (RCW) (concentrate)	12 ml
DNase/RNase-Free Water	6 ml
Nucleopore RCC Columns	50
Collection Tubes	50
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*Please see "Preparation and Storage of reagents"

Reagents, consumables and equipments not provided with the kit

- Ethanol
- Microcentrifuge

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

RNA Binding Buffer

Contain Guanidine Hydrochloride: R&S Phrases: R10-22-36/38, S7-16

R10: Flammable, R22 Harmful by Inhalation, in contact with skin and if swallowed, R36/38: Irritating to eyes and skin. S7: Keep container tightly closed. S16: Keep away from sources of ignition- No Smoking.

Follow the safety guidelines and rules enacted by your research institution or facility.

INTRODUCTION

Principle and Procedure

Nucleopore RNA Clean & Concentrator Kit provides a simple and reliable method for the rapid preparation of up to ~25 µg (per prep.) of high-quality RT-PCR-ready RNA. This simple procedure is based on the use of a unique single-buffer system and Fast-Spin column technology. The procedure is easy: just add the buffer to your sample, adjust the conditions for binding by adding ethanol, and the cleaned RNA is then concentrated into ≥25 µl of RNase-free water using a Nucleopore-RNA Clean and Concentrator (RCC) Column. RNA fragments (≥17 bases) can be safely treated and recovered using this kit. The result is highly-concentrated, purified RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, etc.

Specifications of Nucleopore GDNA Cleanup Kit

- Quick (5 minute) method for cleaning and concentrating RNA.
- Ideal for purification of RNA from aqueous phase following an acid phenol extraction.
- Fast-Spin column technology allows RNA to be eluted into minimal volumes (≥25 µl).
- Eluted RNA is ultra clean and ready for subsequent analysis and molecular manipulation.
- Sample Sources – RNA fragments (≥17 nucleotides): DNase treated RNA, in vitro transcription products, the aqueous phase following an acid phenol extraction methods
- Format – Spin column.
- RNA Purity – High quality RNA (A260/A280 >1.8, A260/A230 >1.8) suitable for all downstream RNA-based manipulations.
- RNA Recovery – Typically, RNA is eluted into as little as 25 µl RNase-free water allowing for a highly concentrated sample. The RNA binding capacity of the supplied RCC Columns is ~25 µg.
- RNA Storage – RNA is eluted with RNase-free water and can be stored at ≤-70 °C.

The addition of RNase inhibitors is optional but highly recommended for prolonged storage.

This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection.

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Preparation and Storage of reagents

Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml RNA Wash Buffer concentrate (R1017)

PROTOCOL

Things to do before starting

Check if wash buffer is prepared as per instructions

Protocol for purification of Total RNA (> 17nt)

1. Add 2 volumes of RNA Binding Buffer (RCB) to each volume of RNA sample and mix well.
2. Add 1 volume ethanol (95-100%) to the mixture from Step 1 (e.g., 200 µl ethanol and 200 µl mixture), and mix well.
3. Transfer the mixture from Step 2 to the Nucleopore-RCC Column in a Collection, Tube and centrifuge at ≥12,000 x g for 1 minute. Discard the flow-through.
4. Add 400 µl RNA Prep Buffer (RCP) to the column and centrifuge at ≥12,000 x g for 1 minute. Discard the flow-through.
5. Add 800 µl RNA Wash Buffer (RCW) to the column and centrifuge at ≥12,000 x g for 30 seconds. Discard the flow-through. Repeat the wash step with 400 µl RNA Wash Buffer.
6. Centrifuge the Nucleopore-RCC Column in an emptied Collection Tube at ≥12,000 x g for 2 minutes. Remove the Nucleopore-RCC Column carefully from the Collection Tube and transfer it into an RNase-free tube.

Add ≥25 µl of DNase/RNase-Free Water directly to the column matrix and let stand for 1 minute at room temperature. Centrifuge at 10,000 x g for 30 seconds. The eluted RNA can be used immediately or stored at -70°C.

Protocol for purification of Total RNA (>200nt)

1. Mix 1 volume of RNA Binding Buffer (RCB) with 1 volume ethanol (95-100%).
2. Add 2 volumes of the adjusted buffer from Step 1 to 1 volume of an RNA sample (e.g., 200 μ l mixture and 100 μ l RNA) and mix well.
3. Transfer the mixture from Step 2 to the Nucleopore-RCC Column in a Collection, Tube and centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.
4. Add 400 μ l RNA Prep Buffer (RCP) to the column and centrifuge at $\geq 12,000 \times g$ for 1 minute. Discard the flow-through.
5. Add 800 μ l RNA Wash Buffer (RCW) to the column and centrifuge at $\geq 12,000 \times g$ for 30 seconds. Discard the flow-through. Repeat the wash step with 400 μ l RNA Wash Buffer (RCW).
6. Centrifuge the Nucleopore-RCC Column in an emptied Collection Tube at $\geq 12,000 \times g$ for 2 minutes. Remove the Nucleopore-RCC Column carefully from the Collection Tube and transfer it into an RNase-free tube.
7. Add $\geq 25 \mu$ l of DNase/RNase-Free Water directly to the column matrix and let stand for 1 minute at room temperature. Centrifuge at 10,000 $\times g$ for 30 seconds. The eluted RNA can be used immediately or stored at -70°C .

Protocol for On-Column DNase Digestion

The DNase digestion procedure can be performed using any source of RNase free DNase I together with its 10X reaction buffer (e.g., 100 U RNase-free DNase I (1U/ μ l) w/ 10X Reaction Buffer. DNase I maintain activity in the RNA Wash Buffer (RCW) provided in this kit.

1. Make the following DNase I cocktail (for each sample to be treated):

RNase-Free DNase I	10 μ l (1 U/ μ l)
10X Reaction Buffer	10 μ l
RNA Wash Buffer (RCW)	80 μ l

2. Following Step 3 of the RNA isolation protocol, add 400 μ l RNA Wash Buffer to the Nucleopore-RCC Column in a Collection Tube and centrifuge at $\geq 12,000 \times g$ for 30 seconds. Discard the flow through.
3. Add 100 μ l DNase I cocktail from Step 1 above directly to the matrix of the Nucleopore-RCC Column. Keep the Nucleopore- RCC Column in the Collection Tube.
4. Incubate the column at 25-37 $^{\circ}\text{C}$ for ≥ 15 minutes, then centrifuge $\geq 12,000 \times g$ for 30 seconds. Discard the flow-through.
5. Continue with Step 4 of the RNA isolation protocol

TROUBLESHOOTING GUIDE

Low Yield

Possible cause

- pH of elution buffer/water too low

Suggestions

- Check the pH of Elution Buffer (1XTE Buffer pH 8.0) or water used for elution. It should be more than 5. Pre-warm elution buffer/water before elution.

Low 260/230 ratio

Possible cause

- Carryover of chaotropic salts

Suggestions

- Give an additional wash step, followed by a dry step at same force and time (step 5).

ORDERING INFORMATION

Description	Pack Size	Cat. No.
® DNASure Tissue Mini Kit	50 preps	NP-61305
® DNASure Plant Mini Kit	50 preps	NP-79105
® DNASure Plant Mini Kit	250 preps	NP-79107
® DNASure Plant Midi Kit	20 preps	NP-78153
® DNASure Plant Maxi Kit	10 preps	NP-78164
® DNASure Blood Mini Kit	50 preps	NP-61105
® DNASure Blood Mini Kit	250 preps	NP-61107
® DNASure Blood Midi Kit	20 preps	NP-61184
® DNASure Blood Maxi Kit	10 preps	NP-61193
® DNASure Blood FastPure Kit	50 preps	NP-62205
® DNASure Blood FastPure Kit	250 preps	NP-62207
® SureSpin Plasmid Mini Kit	50 preps	NP-37105
® SureSpin Plasmid Mini Kit	250 preps	NP-37107
® SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
® SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
® SureSpin Buffer Set*	1	37107-BS
® SurePrep Plasmid Mini Kit	20 preps	NP-15123
® SurePrep Plasmid Mini Kit	100 preps	NP-15125
® SurePrep Plasmid Midi Kit	20 preps	NP-15143
® SurePrep Plasmid Midi Kit	100 preps	NP-15145
® SurePrep Plasmid Maxi Kit	10 preps	NP-15161
® SurePrep Plasmid Maxi Kit	25 preps	NP-15162
® SurePrep Plasmid Mega Kit	5 preps	NP-15183
® SurePrep Plasmid Giga Kit	5 preps	NP-15191

*SureSpin® Buffer Set

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

**SureSpin® Buffer Set

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleopore® RNA Clean & Concentrator Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleopore® RNA Clean & Concentrator Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleopore® RNA Clean & Concentrator Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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