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Animal Tissue RNA Purification Kit — Product Insert

Product # 25700

Norgen's Animal Tissue RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from all types of animal tissue samples, including fiber-rich tissues such as muscle and heart. Norgen's Animal Tissue RNA Purification Kit is provided with Proteinase K, which aids in the removal of the various proteins present in fiber-rich tissues including collagen, contractile proteins and connective tissues. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA), without the use of inhibitory phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the tissue of interest with Buffer RL, followed by treatment with the provided Proteinase K (please see the flow chart on page 4). The sample is then centrifuged, ethanol is added to the supernatant, and the solution is loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations, thus only the RNA will bind to the column while any remaining proteins and other contaminants will be removed in the flowthrough or retained on the top of the resin. At this point, an on-column DNAase digestion is performed to remove any traces of DNA which may co-purify with the RNA. A series of wash steps are then performed to remove the DNase and any remaining impurities, and lastly the purified total RNA is eluted with Elution Solution A. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Heart Kidney Liver Muscle Spleen	 30 mg 15 mg 15 mg 30 mg 15 mg
Time to Complete 10 Purifications	50 minutes
Average Yields* Rat Muscle (10 mg) Rat Liver (10 mg)	 5 µg 30 µg

Storage Conditions and Product Stability

The DNAse I should be stored at -20°C upon arrival. The Proteinase K should be stored at -20°C upon arrival and after reconstitution. All other solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date shipment.

Advantages

- Isolate high quality total RNA from a variety of animal tissues, including fiber-rich tissues
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Fast and easy processing using rapid spin-column format
- On-column DNAse treatment for removal of any contaminating DNA

Kit Components

Component	Product # 25700 (50 preps)
Buffer RL	30 mL
RNase-Free Water	40 mL
Wash Solution A	38 mL
Enzyme Incubation Buffer A	6 mL
Elution Solution A	6 mL
Proteinase K	2 vials
DNase I	1 vial
Mini Spin Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	50
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Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Tissue of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Animal Tissue RNA Purification Kit:

- Benchtop microcentrifuge
- 96 - 100% ethanol
- β-mercaptoethanol
- Liquid nitrogen
- Mortar and pestle
- 55°C incubator

Flowchart

Procedure for Purifying Total RNA using Norgen's Animal Tissue RNA Purification Kit

Excise tissue sample. Add **Buffer RL** and homogenize.

Add Proteinase K and incubate

SPIN

Add Ethanol

Bind to column.
Wash once with Wash Solution A

SPIN

Add DNaseI and incubate

SPIN

Wash twice
with Wash Solution A

SPIN

Elute RNA with
Elution Solution A

SPIN

Purified Total RNA

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Procedures
All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- All enzymes provided should remain at the storage temperature indicated on each vial until use.
- Reconstitute each of the provided **Proteinase K** vials in 600 µL of molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free), aliquot into small fractions and store the unused portions at -20°C until needed.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Buffer RL** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.

- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to grinding with the mortar and pestle.
- Tissues stored in RNA stabilization reagents such as RNA*later*® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry any excessive liquid.
- It is very important not to exceed the recommended starting amount for each tissue. Please refer to Table 1 below for the recommended maximum input amounts of each tissue.

Table 1. Recommended Maximum Input Amounts of Different Tissues

Tissue	Maximum Input Amount
Heart	30 mg
Kidney	15 mg
Liver	15 mg
Muscle	30 mg
Spleen	15 mg

1. Cell Lysate Preparation

a. Excise the tissue sample from the animal.
b. Determine the amount of tissue by weighing. Please refer to Table 1 for the recommended maximum input amounts of different tissues. **For tissues not included in the table, we recommend starting with an input of no more than 10 mg.**
c. Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.

Note: The use of liquid nitrogen is recommended. However, if homogenization without flash-freezing is preferred, proceed to Step **1e**.

d. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
e. Add 300 µL of **Buffer RL** to the tissue sample and continue to grind until the sample has been homogenized.

Note: Maximum homogenization may be achieved by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

f. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).
g. Add 600 µL of **RNase-Free Water** (provided) to the lysate. Vortex to mix.
h. Add 20 µL of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.
i. Spin the lysate for 1 minute to pellet any cell debris. Transfer the supernatant to a new RNase-free microcentrifuge tube (not provided).
j. Add 450 µL of 96 - 100% ethanol (provided by the user) to the lysate. Vortex to mix.

2. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 650 μL of the lysate with the ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **14,000 x *g* (~14,000 RPM)**.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step **2b** and **2c** as necessary.

Note: If part of the lysate has not passed into the collection tube after Step **2d** and the volume is less than 200 μL, continue to Step **2e** without additional centrifugation.

e. Apply 400 μL of **Wash Solution A** to the column and centrifuge for 2 minutes.
f. Discard the flowthrough and assemble the spin column with a new collection tube.

3. On-Column DNAse Treatment

Optional: If DNase Treatment is not required, proceed directly to Step 4a of Column Wash.

a. Apply 100 μL of **Enzyme Incubation Buffer A** and 15 μL of **DNase I** to the column and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure that the entire 115 μL of DNase I mix passes through the column. If needed, spin at 14,000 x g (~14,000 RPM) for an additional minute.

b. After the centrifugation in Step 3a, pipette the flowthrough that is present in the collection tube back onto the top of the column.

Note: Ensure Step 3b is performed in order to ensure maximum DNase activity and to obtain maximum yield of RNA, in particular for small RNA species.

c. Incubate at room temperature for 15 minutes.

4. Column Wash

a. Apply 400 μL of **Wash Solution A** to the column containing the DNase I mix and centrifuge for 1 minute.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **4a** and **4b** to wash column a second time.
d. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Solution A** to the column.

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b** and **5c**).

6. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in tissue used	Different tissues have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Insufficient solubilization of tissues	Ensure the lysate is diluted with the appropriate amount of RNase-free water, and that the appropriate amount of Proteinase K is added. Also ensure that the Proteinase K treatment is performed at 55°C for the full 15 minutes. The incubation time can be increased up to 30 minutes if required.

Problem	Possible Cause	Solution and Explanation
Clogged Column Clogged Column	Insufficient solubilization of tissues	Ensure the lysate is diluted with the appropriate amount of RNase-free water, and that the appropriate amount of Proteinase K is added. Also ensure that the Proteinase K treatment is performed at 55°C for the full 15 minutes. The incubation time can be increased up to 30 minutes if required.
	Maximum amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column. Also, ensure that the on-column DNase treatment is performed if high amounts of genomic DNA are found in the sample.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Frozen tissues were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed 2 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 2 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform the RNAse-free DNaseI digestion on the RNA sample suggested in the protocol to remove genomic DNA contamination.
	DNase I mix did not completely pass through the column during DNase I treatment	Ensure the entire 115 µL of DNase I mix passes through the column. If needed, spin at 14,000 x g (~14,000 rpm) for 1 minute

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

Technical Support
Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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