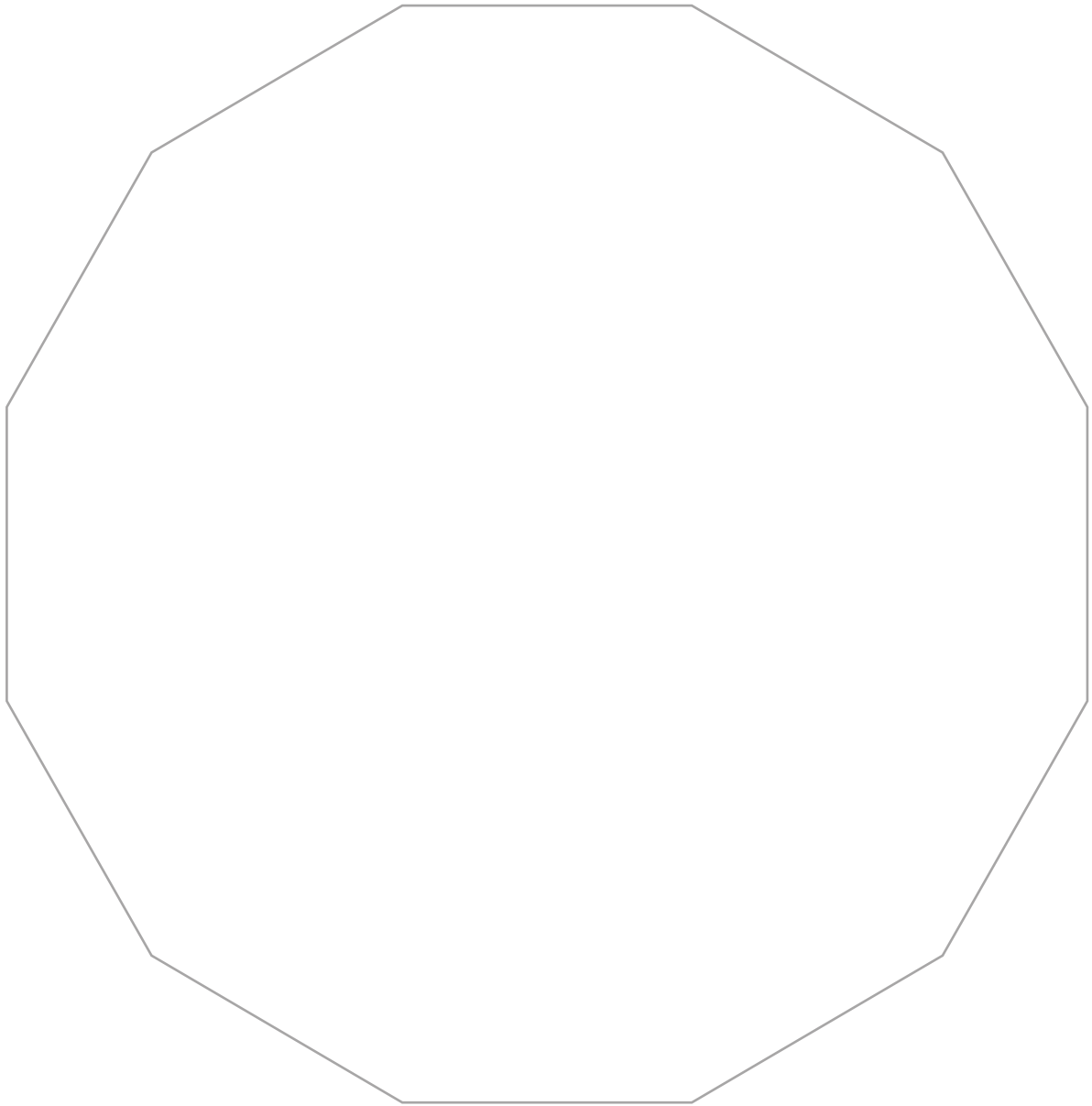
TOTAL RNA EXTRACTION SYSTEM
PROTOCOL & TECHNICAL BULLETIN



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KIT CONTENTS

50 RXN Kit (TRE0001S):

ITEM	CATALOG #	50 RXN
RNA Extraction Buffer	TRE0002	50ml
Wash Buffer Concentrate	TRE0004	12ml
NucleoPur™ Spin Columns	TRE0006	50 columns
Collection Tubes	CT0050	50 tubes

250 RXN Kit (TRE0001L):

ITEM	CATALOG #	250 RXN
RNA Extraction Buffer	TRE0003	2 bottles/125ml
Wash Buffer Concentrate	TRE0005	2 bottles/24ml
NucleoPur™ Spin Columns	TRE0007	250 columns
Collection Tubes	CT0250	250 tubes

PRECAUTIONS

THIS PRODUCT IS NOT INTENDED OR APPROVED FOR USE IN HUMANS OR VETERINARY ANIMALS. THE USE OF THIS PRODUCT IN A THERAPEUTIC SETTING IS HAZARDOUS AND MAY RESULT IN ILLNESS, INJURY AND/OR DEATH.

Please read these instructions carefully before using this system.

The reagents in this system have been formulated and tested to work exclusively with the BenevBio Total RNA Extraction System. This system may not perform as described if any reagent or procedure is replaced and/or modified.

For research use only. Not for human or diagnostic use.

WARRANTY

BenevBio guarantees the performance of all products when used as directed for their intended purpose. Should any product fail to perform satisfactorily for any reason other than misuse, BenevBio's sole liability hereunder shall be limited to refund of the purchase price or, at the discretion of BenevBio, the replacement of all material that does not meet our specifications. BenevBio shall not be liable otherwise or for incidental or consequential damages including, but not limited to, the cost of handling. We reserve the right to change or modify any components to enhance the performance or design. The Buyer must give notice within thirty (30) days after receipt of material or shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

BenevBio CUSTOMER CARE INFORMATION

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STORAGE AND STABILITY

The system will perform as specified if stored dry at room temperature (20-25°C). Under these conditions the kit can be stored for up to 6 months without any decline in performance and/or quality.

ADDITIONAL ITEMS REQUIRED

1. Microcentrifuge capable of reaching 16,000 x g.
2. Adjustable pipettor.
3. Ethanol.
4. Microcentrifuge tubes.
5. RNase free tips, tubes, etc.
6. A source of "UltraPure" water. Water used to elute samples must be deionized and free of trace organic contaminants.

Note: UltraPure water is available for purchase.

INTRODUCTION AND DESCRIPTION

The BenevBio Total RNA Extraction System is designed to extract high quality ready-to-use RNA from tissues and cells in as little as 15 minutes. The NucleoPur™ Columns are specifically designed to provide the highest quality RNA with minimum copurification of DNA.

Samples are efficiently recovered and concentrated in as little as 35µl for sensitive applications including RT-PCR, qRT-PCR, cDNA synthesis, Northern blot, and microarray analysis.

PROTOCOL

NOTE:

1. Be sure to add β-Mercaptoethanol to the RNA Extraction Buffer to make a final concentration of 1%. For example, add 250ul β-Mercaptoethanol to 25mls RNA Extraction Buffer.
2. Add an appropriate volume of ethanol (95-100%) to the Wash Buffer Concentrate before use. See bottle label for volume.

Tissues:

1. Place up to 20mg of fresh tissue in 400ul RNA Extraction buffer. Homogenize completely on ice.
   Incomplete homogenization may result in lower than expected yields as well as clogging of the column.
2. Carefully remove the supernatant and transfer to a spin column.
3. Close the lid and place the NucleoPur™ column into a 2ml collection tube.
4. Centrifuge at 10,000 x g for 1 minute. If column appears clogged due to incomplete homogenization centrifuge at higher speeds (up to 16,000 x g for 1 minute).
5. Discard the flow-through.
6. Add 400ul wash buffer into the spin column and centrifuge at 10,000 x g for 1 minute.
7. Discard the flow-through.

(OPTIONAL) **DNase Digestion see page 12.**

8. Repeat steps 6 and 7 except spin at 16,000 x g for one minute.
9. Place the NucleoPur™ spin column into a 1.5ml centrifuge tube.
10. Add 35-50uls RNase-free water directly to the column matrix and spin at 16,000 x g for 1 minute.
11. (OPTIONAL). Either add another 35-50uls RNase-free water directly to the column matrix and spin at 16,000 x g for 1 minute or use the original eluate from step 10 to the NucleoPur™ column to achieve a higher final concentration.
12. Use samples immediately or store at -80C for future use.

Cell culture:

Do not use more than 1 x 10E7 cells whether in suspension or monolayer.

Suspension:
Pellet cells by centrifugation for 5 min at 300 x g.
Discard supernatant.

1. Pipet 400uls RNA Extraction Buffer into the microcentrifuge tube containing cell pellet.
2. Briefly pulse vortex to completely disrupt the pellet.

PROTOCOL

3. After pipeting up and down to ensure mixing of the cells transfer the mixture to a NucleoPur™ spin column.
4. Close the lid and place the NucleoPur™ column into a 2ml collection tube.
5. Centrifuge at 10,000 x g for 1 minute.

Note: Column may clog if more than 1 x 10E7 cells are used. If this occurs centrifuge at higher speeds (up to 16,000 x g for 1 minute).

6. Discard the flow-through.
7. Add 400ul wash buffer into the spin column and centrifuge at 10,000 x g for 1 minute.
8. Discard the flow-through.

(OPTIONAL) **DNase Digestion See Page 12.**

9. Repeat steps 6 and 7 except spin at 16,000 x g for 1 minute.
10. Place the NucleoPur™ spin column into a 1.5ml centrifuge tube.
11. Add 35-50uls RNase-free water directly to the column matrix and spin at 16,000 x g for 1 minute.
12. (OPTIONAL). Add another 35-50uls RNase-free water directly to the column matrix and spin at 16,000 x g for 1 minute or use the original eluate from step 10 to the NucleoPur™ column to achieve a higher final concentration.
13. Use samples immediately or store at -80C for future use.

Monolayer:

Do not use more than 1 x 10E7 cells.

1. Aspirate the medium and wash cells with PBS.
2. Place the culture dish on ice and pipet 1ml of RNA Extraction Buffer directly onto the dish and pipet the mixture up and down. One may optionally scrape the cells off the plate.

Note: If the mixture of cells and buffer is too viscous add another 1ml of RNA Extraction Buffer to the dish. Viscous samples may clog the column and decrease yield.

3. After the cells have detached, pipet mixture into a NucleoPur™ Spin Column.

Note: The column can hold up to 700uls at a time.

4. Proceed with Step #4 on page 10.

PROTOCOL

DNase Treatment of Samples:

Optional DNase treatment of RNA samples:
The Total RNA extractions yield high quality RNA, but some DNA may co-purify with the RNA that may interfere with sensitive down stream procedures such as qRT-PCR.

DNase treatment AFTER first wash step (Step#7 in tissue protocol or Step #8 in cell protocol).

The DNase digestion reaction is as follows:

10x Reaction Buffer	8ul
DNaseI (1unit/ul)	5ul
RNase free water	67ul
Total rxn volume	80ul per sample

1. Add DNase digestion reaction (80ul) directly to the column and incubate at 25-37C for 15 minutes.
2. Centrifuge at 10,000 x g for 1 minute.
3. Continue with Step #8 in tissue protocol or Step #9 in cell protocol).

TROUBLESHOOTING

Low RNA yield:

Insufficient homogenization.	Ensure complete homogenization of starting material.
Too much starting material.	Either decrease amount of starting material or increase volume of RNA Extraction Buffer.
Ethanol carryover.	During the second wash step be sure to centrifuge samples at 16,000 x g for 1 minute and carefully remove the column from the collection tube so the column does not come in contact with flow-through. If necessary, place column in a new collection tube and perform the centrifugation.
Clogged column.	Insufficient homogenization may lead to lower than expected yields.

TROUBLESHOOTING

RNA degraded:	1. Be sure to use RNase free pipet tips, tubes, etc. when working with RNA. 2. If using fresh tissue or cells be sure to immediately homogenize in RNA Extraction buffer on ice.
DNA Contamination:	
Too much starting material.	DNA may co-precipitate with the RNA if too much starting material is used. Be sure to use less starting material in subsequent experiments.
Did not perform DNase digestion.	One may perform an on-column DNase digestion to minimize DNA contamination of RNA preparations.

RELATED PRODUCTS

ITEM	CATALOG #	SIZE
DNA free RNA System	DRS0001S	50 RXN
Viral RNA Extraction System	VRE0001S	50 RXN
DNase I w/ 10x Buffer	DRS0006	100 units

ORDERING INFORMATION

BenevBio
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