Product Information

ExoBrite™ EV Total RNA Isolation Kit

Catalog Number: 28001 (50 preps)

Kit Contents

Component	Size
99885: EV Lysis Buffer	36 mL
99886: Column Binding Buffer	15 mL
99865: DNase Buffer	5 mL
99867-3000U: DNase I (lyophilized)	3000 U (Reconstitute in 120 uL water prior to use)
99887: Column Wash Buffer	12 mL (Add 48 mL ethanol prior to use)
99888-50: Spin Columns	50 each

Storage and Handling

Upon receipt, store DNase I at 2-8°C. After reconstitution, DNase I solution should be stored at -20°C. Store other kit components at room temperature. Kit components are stable for at least 12 months from date of receipt when stored as recommended. The EV Lysis Buffer and Column Binding Buffer contain chaotropic guanidine salts, which are hazardous (see SDS). Use gloves and other appropriate laboratory protection when using this kit. Mixing bleach with guanidine salts can produce hazardous byproducts. DO NOT mix waste from this kit with bleach. Handle all kit components using universal laboratory safety precautions.

Product Description

This kit was designed for efficient isolation of total RNA from extracellular vesicles (EVs). The reagents and protocol for this kit have been optimized to extract RNA of any size, including mRNA and miRNA, from EVs. The isolated EV RNA can then be used for downstream analysis such as qPCR or RNAseq. The RNA isolation procedure is a simple column purification method that takes as little as 20 minutes and requires no phenol/chloroform or ethanol precipitation steps. The amount of RNA recovered will depend primarily on EV number and quality, which may be affected by purification method, storage conditions, and number of freeze thaws (see “Assessing RNA quantity and quality “on page 2). An optional, but recommended, DNase treatment step is used to remove contaminating DNA.

Experimental Protocols

Materials required but not supplied

- Ethanol (200 proof, molecular biology grade)
- 1.5 mL microcentrifuge tubes (Eppendorf LoBind® tubes recommended)
- Microcentrifuge (12,000 x g minimum)
- RNase-free water for DNase I reconstitution and final RNA elution step (see Related Products for Water, Ultrapure Molecular Biology Grade)

Before you begin

Prepare the following buffers

- Column Wash Buffer: Add 48 mL of 200 proof ethanol to the bottle and mix well. Mark the ethanol added box on the label.
- DNase I: Reconstitute the lyophilized DNase I by adding 120 uL of RNase-free water. Using a pipette, mix gently to ensure DNase is fully reconstituted (do not vortex, which can denature DNase). Briefly centrifuge the tube to collect contents at bottom. Store in aliquots at -20°C and avoid freeze/thaw cycles.

Experimental Protocols, continued

1. Sample lysis

The ExoBrite™ EV Total RNA Isolation Kit can be used with EVs from a variety of fresh or fresh/frozen sources.

If using EVs in solution:

Use a starting volume of up to 400 uL of EVs. For best results, use the most EVs that you can. If possible, determine the concentration of EVs in your sample to best predict the RNA yield. In our experience with EVs from cultured cells isolated by SEC, we expect ~ 10-20 ng of RNA from $1x10^{10}$ EVs. Yields from different EV sources may vary.

If using bead-bound EVs:

Remove the solution from the beads (i.e., by a magnet or centrifugation, as appropriate). Resuspend the beads in 100 uL EV Lysis Buffer and vortex. Remove the beads (by magnet or centrifugation as appropriate) and transfer the lysate to a fresh tube.

2. RNA isolation

2.1 Add ethanol to the lysate to 55% final concentration and vortex to mix. For example, to 100 uL of lysate add 120 uL of 200 proof ethanol, or to 400 uL lysate add 480 uL ethanol. Do not centrifuge. Immediately proceed to the following step.

2.2 Transfer up to 700 uL of the sample, including any precipitate, to a spin column. Centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

2.3 Repeat Step 2.2 until the entire sample volume has passed through the spin column.

3. DNase I treatment (recommended)

Note: This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 4.1.

3.1 Mix 240 uL of Column Binding Buffer and 360 uL of 100% ethanol (200 proof) in a separate tube, for a total volume of 600 uL.

3.2 Add 300 uL of this mixture to the spin column. Centrifuge for 30 seconds at 16,000 x g and discard the flow-through.

3.3 Mix 70 uL of DNase Buffer with 2 uL of reconstituted DNase I, and add this mixture directly to the center of the membrane of the spin column. Let stand for 15 minutes at room temperature.

3.4 Add the remaining 300 uL of the Column Binding Buffer/ethanol mixture (prepared in step 3.1) to the column. Centrifuge for 30 seconds at 16,000 x g and discard the flow-through.

4. Continue RNA isolation

4.1 Add 500 uL of Column Wash Buffer to the spin column. Close the tube lid, and centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

4.2 Wash again by repeating step 4.1.

4.3 Dry the spin column by placing it back into an emptied collection tube and centrifuging again for 3 minutes at 16,000 x g. Discard the flow-through. Place the spin column in a clean microcentrifuge tube.

4.4 Elute the pure RNA by adding 50 uL of nuclease-free water to the center of the membrane of the spin column. Let stand for 1 minute. Centrifuge for 1 minute at 16,000 x g. Collect the eluted RNA from the centrifuge tube.

Note: RNA can be eluted in volumes as low as 30 uL. The concentration will be higher but total yield may be lower.

4.5 Optional: Repeat step 4.4 for a higher RNA yield but at a lower concentration.

4.6 Eluted RNA should be stored at -80°C.

Assessing RNA quantity and quality

The amount of RNA recovered may be affected by EV purification method, storage conditions, and number of freeze thaws. However, we normally expect ~ 10-20 ng of RNA from $1x10^{10}$ SEC-isolated EVs. RNA quantification kits, such as our AccuBlue® RNA Quantitation Kit, or systems such as the Bioanalyzer® or Fempto Pulse®, can be used to measure the quality and quantity of RNA purified from EV samples. Running the RNA samples on an agarose gel using our EMBER™ Ultra RNA Gel Kit and imaging using the Gel-Bright™ Laser Diode Gel Illuminator or other blue LED illuminator provides a simple and sensitive way to check RNA quality by gel electrophoresis.

Related Products

Cat. No.	Product
41024-4L	Water, Ultrapure Molecular Biology Grade
41032	EMBER500™ RNA Prestain Loading Dye
41044	EMBER™ Ultra RNA Gel Kit
31073	AccuBlue® Broad Range RNA Quantitation Kit
22028	RNase-X™ Decontamination Solution
E90005	Gel-Bright™ Laser Diode Gel Illuminator
30129-30130	ExoBrite™ True EV Membrane Stains
30111-30114	ExoBrite™ CTB EV Staining Kits
30119-30122	ExoBrite™ Annexin EV Staining Kits
30123-30126	ExoBrite™ WGA EV Staining Kits
30115-30118	ExoBrite™ STORM CTB EV Staining Kits
30127	ExoBrite™ EV Surface Stain Sampler Kit, Green
28000	ExoBrite™ Streptavidin Magnetic Beads
CD507	CELLDATA DNAstorm™ 2.0 FFPE DNA Extraction Kit
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P003-410... P003-650	ExoBrite™ CD9 Flow Antibodies
P004-410... P004-560	ExoBrite™ CD63 Flow Antibodies
P005-410... P005-560	ExoBrite™ 410/450 CD81 Flow Antibody
P008-410... P008-650	ExoBrite™ IgG1 Isotype Control Flow Antibodies

Please visit our website at www.biotium.com for information on our products for EV research, including unique EV surface stains and optimized tetraspanin antibodies for flow cytometry or western blotting.