# Product Information

## ExoBrite™ EV Total RNA Isolation Kit

**Catalog Number:** 28001

### Kit Contents

| Component | Size |
|---|---|
| 99885: EV Lysis Buffer | 36 mL |
| 99886: Column Binding Buffer | 15 mL |
| 99865: DNase Buffer | 5 mL |
| 99867-600U: DNase I (Lyophilized) | 2 vials |
| 99887: Column Wash Buffer | 12 mL |
| 99888-50: Spin Columns | 50 each |

**Unit Size:** 50 preps

### Storage and Handling

Upon receipt, store DNase I (Lyophilized) at 4°C. After reconstitution, store DNase I solution at -20°C. Store all 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

The ExoBrite™ EV Total RNA Isolation Kit was designed for efficient isolation of total RNA from extracellular vesicles (EVs) and can be used with EVs from a variety of fresh or fresh/frozen sources. 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 the purification method, storage conditions, and number of freeze/thaws (see “Assessing RNA quantity and quality” on page 2).

The protocol includes two optional but recommended DNase treatments to remove contaminating DNA. For vesicles bound to EV antibody capture beads (i.e., bead-bound EVs), the DNase pre-treatment is recommended to remove externally bound DNA prior to EV lysis and RNA isolation. An on-column DNase treatment is recommended for all samples. For bead-bound EVs, DNase pre-treatment improves RNA yield compared to on-column treatment alone, and combined pre-treatment and on-column treatment of bead-bound EV samples gives the best DNA removal. Although either DNase treatment alone removes contaminating DNA from bead-bound EVs, RNA yield is reproducibly higher when both the pre-treatment and on-column treatment are performed (see App Note: DNase pre-treatment of the EV exterior improves EV RNA yield).

### Experimental Protocol

**Materials required but not provided**

- 100% ethanol (molecular biology grade)
- 1.5 mL microcentrifuge tubes (Eppendorf LoBind® tubes recommended)
- RNase-free water (see Related Products)

**Prepare the following buffers**

- Column Wash Buffer: Add 48 mL of 100% ethanol to the bottle and mix well. Mark the ethanol added box on the label.
- DNase I: Reconstitute a vial of the lyophilized DNase I by adding 60 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.

**1. DNase pre-treatment for bead-bound EVs (optional)**

1.1 Remove the solution from the beads (i.e., by a magnet or centrifugation, as appropriate) and wash the beads with PBS.

1.2 Resuspend the beads in 70 uL of DNase Buffer. Add 2 uL of DNase I and incubate for 15 minutes at room temperature, rotating.

1.3 Wash the beads three times with PBS.

**2. Sample lysis**

2.1 If using EVs in solution: Use a starting volume of up to 400 uL of EVs. Add an equal volume of EV Lysis Buffer to the EV sample (up to 400 uL Lysis Buffer for up to a total volume of 800 uL sample + Lysis Buffer).

**Note:** For best results, use as many EVs as you can. If possible, determine the concentration of EVs in your sample to best predict the RNA yield. We typically expect ~10-20 ng of RNA from $1x10^{10}$ SEC-isolated EVs. Yields from different EV sources may vary. See Tech Tip: Isolation and Staining of Extracellular Vesicles for information on EV isolation.

2.2 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.

**3. RNA isolation**

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

3.2 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.

3.3 Repeat step 3.2 until the entire sample volume has passed through the spin column.

**4. DNase I treatment (recommended)**

**Note:** This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 5.1.

4.1 Mix 240 uL of Column Binding Buffer and 360 uL of 100% ethanol in a separate tube, for a total volume of 600 uL.

4.2 Add 300 uL of the Column Binding Buffer/ethanol mixture to the spin column. Centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

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

4.4 Add the remaining 300 uL of the Column Binding Buffer/ethanol mixture from step 4.1 to the column. Centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

**5. Continue RNA isolation**

5.1 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.

5.2 Wash again by repeating step 5.1.

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

5.4 Elute the pure RNA by adding 50 uL of RNase-free water to the center of the spin column membrane. 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.

5.5 Optional: Repeat step 5.4 for a higher RNA yield but at a lower concentration.

5.6 Store eluted RNA at -80°C.

## Assessing RNA Quantity and Quality

The amount of RNA recovered may be affected by the EV purification method, storage conditions, and number of freeze/thaws. We normally expect ~10-20 ng of RNA from $1x10^{10}$ SEC-isolated EVs. RNA quantitation kits, such as our AccuBlue® Broad Range RNA Quantitation Kit (Cat. No. 31073), 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 (Cat. No. 41044) and imaging using the Gel-Bright™ Laser Diode Gel Illuminator (Cat. No. E90005) 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... 30137 | 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 |
| CD506 | CELLDATA RNAstorm™ 2.0 FFPE RNA Extraction Kit |
| CD508 | CELLDATA DNAstorm™/RNAstorm™ 2.0 Combination Kit |
| CD504 | CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit |
| P003-410... P003-RPE | ExoBrite™ CD9 Flow Antibody |
| P018-410... P018-APC | ExoBrite™ CD9 (Mouse) Flow Antibody |
| P004-410... P004-RPE | ExoBrite™ CD63 Flow Antibody |
| P022-410... P022-APC | ExoBrite™ CD63 (Mouse) Flow Antibody |
| P005-410... P005-RPE | ExoBrite™ CD81 Flow Antibody |
| P019-410... P019-APC | ExoBrite™ CD81 (Mouse/Rat) Flow Antibody |
| P061-410... P061-640 | ExoBrite™ CD47 Flow Antibody |
| P040-410... P040-640 | ExoBrite™ CD29 Flow Antibody |
| P008-410... P008-RPE | ExoBrite™ IgG1 Isotype Control Flow Antibody |
| P056-410... P056-APC | ExoBrite™ Armenian Hamster IgG Isotype Control Flow Antibody |
| P057-410... P057-APC | ExoBrite™ Rat IgG2a Isotype Control Flow Antibody |
| P030-P032 | ExoBrite™ CD9/CD63/CD81 Single-Color Antibody Cocktails |
| P028, P029 | ExoBrite™ CD9/CD63/CD81 3-Color Antibody Cocktails |

Please visit our website at www.biotium.com for information on our products for EV detection, including EV membrane stains, antibodies for flow cytometry and western blotting, and antibodies and stains for STORM.

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