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EV RNA/mRNA Isolation

Supplement Protocol for the EVs360™ Multi-Omics Kit

START POINT Begin only after EV capture is complete and the EV-bound magnetic beads have been collected on the magnetic stand.

Before you begin

Workflow	Recommended kit	Catalog number
EV RNA/mRNA purification	Zymo Research Quick-cfRNA™ Serum & Plasma Kit	R1059

RNASE CONTROL Use RNase-free tubes, pipette tips, and water. Keep the work area and gloves clean, and minimize unnecessary handling.

Default centrifugation setting

Unless a step states otherwise, centrifuge at 12,000 × g for 30 seconds at room temperature (20–30°C).

PHASE 1 Prepare the lysis reaction

Reagent	Volume per reaction	Operator note
Quick-cfRNA™ Digestion Buffer	200 µL	Add directly to the EV-bound beads.
Protease K	10 µL	Add directly to the same reaction tube.

This workflow was internally validated with the kit above. Follow applicable laboratory safety procedures and the current reagent manufacturer instructions.

PHASE 1 Lyse and clear the sample

1	**ADD LYSIS REAGENTS** Add 200 µL Quick-cfRNA™ Digestion Buffer and 10 µL Protease K directly to each EV-bound bead reaction.
2	**MIX** Vortex for 15 seconds, then briefly centrifuge.
3	**DIGEST** Incubate at 37°C for 30 minutes.
4	**ADD BINDING BUFFER** Add 400 µL Quick-cfRNA™ Binding Buffer to the digested sample. Mix thoroughly by vortexing.
5	**MAGNETICALLY SEPARATE** Place the tube on the magnetic stand. Transfer the cleared lysate to a new RNase-free tube; leave the magnetic beads behind.

CRITICAL TRANSFER Keep the sample tube on the magnet while transferring the lysate. Do not transfer magnetic beads.

PHASE 2 Remove larger contaminants

6	**ADD ISOPROPANOL** Add 1 mL of 100% isopropanol to the cleared lysate. Mix thoroughly by vortexing.
7	**LOAD THE FILTER** Load 600 µL onto the Spin-Away™ Filter and centrifuge using the default setting.
8	**REPEAT LOADING** Repeat the 600 µL load and centrifugation two more times so the entire sample, approximately 1.8 mL, passes through the Spin-Away™ Filter.
9	**MOVE AND DRY SPIN** Transfer the Spin-Away™ Filter to a new collection tube and centrifuge for 2 minutes.

PHASE 3 Recover and bind the RNA

10	**RECOVER RNA** Add 600 µL RNA Recovery Buffer directly to the Spin-Away™ Filter. Incubate for 3 minutes, then centrifuge. Save the flow-through.

SAVE THIS FRACTION The flow-through contains the recovered RNA. Do not discard it.

11	**ADD ETHANOL AND LOAD** Add 300 µL ethanol (95–100%) to the saved flow-through. Mix by pipetting, transfer to a Zymo-Spin™ IC Column in a new collection tube, centrifuge, and discard the new flow-through.

PHASE 4 Wash and elute the RNA

12	**WASH SEQUENTIALLY** Wash the Zymo-Spin™ IC Column in the specified order. Wash 1: 400 µL RNA Prep Buffer; centrifuge and discard flow-through • Wash 2: 700 µL RNA Wash Buffer; centrifuge and discard flow-through • Wash 3: 400 µL RNA Wash Buffer; centrifuge for 2 minutes and discard flow-through
13	**ELUTE** Transfer the column to a clean RNase-free tube. Add 15 µL DNase/RNase-Free Water directly to the column matrix, incubate for 2 minutes, and centrifuge.
14	**USE OR STORE** Use the eluted RNA immediately for molecular applications or store at ≤−80°C for future use.

LOW-YIELD SAMPLES For low-yield plasma or serum samples, quantify RNA using the Qubit™ High-Sensitivity RNA Assay.

Completion checklist

- RNase-free consumables were used throughout.
- Magnetic beads remained behind during lysate transfer.
- All approximately 1.8 mL of sample passed through the Spin-Away™ Filter.
- The Step 10 flow-through was saved and used in Step 11.
- All three Zymo-Spin™ IC Column washes were completed in order.
- RNA yield and storage location were documented.

Adapting the workflow

This is an example RNA extraction workflow based on internal validation. EVs captured on magnetic beads may be adapted to other RNA-isolation methods by adding magnetic-stand separation after lysis.