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EV DNA 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 DNA purification	Zymo Research Quick-DNA Microprep Plus Kit	D4074

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

PHASE 1 Prepare the lysis reaction

Reagent	Volume per reaction	Operator note
BioFluid & Cell Buffer	200 µL	Add directly to the EV-bound beads.
Proteinase K	20 µL	Stock concentration: 20 mg/mL. Equivalent Proteinase K from another vendor may be used.

1	**ADD LYSIS REAGENTS** Add 200 µL BioFluid & Cell Buffer and 20 µL Proteinase K to each EV-bound bead reaction.
2	**MIX** Vortex for 15 seconds, then briefly centrifuge.
3	**DIGEST** Incubate at 55°C for 10 minutes.
4	**ADD BINDING BUFFER** Add 220 µL Genomic Binding Buffer (GBB).
5	**MIX AGAIN** Vortex thoroughly, then briefly centrifuge.
6	**MAGNETICALLY SEPARATE** Place the tube on the magnetic stand to separate the beads from the lysate.

CRITICAL TRANSFER In the next step, transfer the cleared lysate—not the magnetic beads—to the Zymo IC-XM Column. Keep the tube on the magnet while pipetting.

PHASE 2 Bind and wash the DNA

7	**LOAD THE COLUMN** Transfer the cleared lysate to a Zymo IC-XM Column in a collection tube. Centrifuge at ≥12,000 × g for 1–1.5 minutes; discard the flow-through.
8	**WASH SEQUENTIALLY** Load and centrifuge after each wash using the same centrifugation setting. Wash 1: 200 µL DNA Pre-Wash Buffer • Wash 2: 500 µL g-DNA Wash Buffer • Wash 3: 200 µL g-DNA Wash Buffer
9	**DRY SPIN** Centrifuge the column for 1–2 minutes to remove residual wash buffer.
10	**MOVE TO A CLEAN TUBE** Transfer the column to a clean collection tube.

PHASE 3 Elute the DNA

11	**ADD ELUTION BUFFER** Apply 6–20 µL DNA Elution Buffer directly to the filter insert. Confirm that the filter is visibly wet.
12	**INCUBATE** Incubate for 5 minutes at room temperature.
13	**ELUTE** Centrifuge at maximum speed, up to 12,000–16,000 × g, for 2 minutes.
14	**USE OR STORE** Use the eluted DNA immediately for molecular applications or store at ≤−20°C for future use.

LOW-YIELD SAMPLES For low-yield samples such as plasma, quantify DNA using the Qubit™ High-Sensitivity DNA Assay.

Completion checklist

- Magnetic beads remained behind during lysate transfer.
- All three column washes were completed in the specified order.
- A dry spin was completed before elution.

- Elution buffer was applied directly to and visibly wetted the filter.
- The DNA concentration method and storage location were documented.

Adapting the workflow

This is an example DNA extraction workflow based on internal validation. EVs captured on magnetic beads may be adapted to other DNA-isolation methods by adding magnetic-stand separation after the lysis step, as shown in Step 6.

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