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EV Protein Extraction

Supplement Protocol for the EVs360™ Multi-Omics Kit

PRODUCT CONFIGURATIONS EVs360™ Multi-Omics Kit, Cat. no. L10001 · 10 rxn | Cat. no. L10002 · 25 rxn

START POINT Begin after EV capture and all three washes are complete. The EV-bound magnetic beads should be free of residual Wash Buffer.

Before you begin

- Use Protein LoBind tubes from capture through lysate recovery.
- Keep samples on ice except during vortexing.
- Prepare lysis buffer fresh when required; add protease and phosphatase inhibitors immediately before use if needed for the downstream assay.
- Use one lysis condition consistently across all samples in a study.

PHASE 1 Choose the lysis buffer

Lysis option	Starting composition	Best suited for
1× RIPA	0.05 M Tris-HCl, pH 7.4; 0.15 M NaCl; 0.25% deoxycholic acid; 1% NP-40; 1 mM EDTA	Western blotting, ELISA, and detergent-compatible immunoassays.
Assay-specific buffer	Use the composition required by the selected platform.	Olink®, NuLISA™, multiplex assays, or other platforms after compatibility validation.

BUFFER COMPATIBILITY Confirm that the lysis buffer is compatible with the protein assay, immunoassay, cleanup method, digestion chemistry, and instrument workflow before processing the study cohort.

PHASE 2 Prepare each reaction

Material	Amount per reaction	Operator note
EV-bound magnetic beads	washed EV-bound magnetic beads	Keep the beads in the same Protein LoBind tube.
Selected lysis buffer	100 µL	Scale only after validating bead resuspension, extraction efficiency, and assay input.

This supplement provides a standardized starting workflow. Application-specific optimization may be required for low-abundance targets, or platform-specific input requirements.

PHASE 3 Lyse the EVs

1	**ADD LYSIS BUFFER** Add 100 µL of the selected lysis buffer directly to the washed EV-bound beads.
2	**RESUSPEND** Vortex vigorously for 1 minute until the bead pellet is completely dispersed.
3	**CHILL** Place the tube on ice for 5 minutes.
4	**REPEAT** Repeat the 1-minute vortex and 5-minute ice cycle two more times. Complete three vortex-ice cycles; total processing time is approximately 20 minutes.

PHASE 4 Recover the protein lysate

5	**MAGNETICALLY SEPARATE** Place the tube on the magnetic stand until the beads are fully collected.
6	**TRANSFER LYSATE** Keeping the tube on the magnet, transfer the cleared protein lysate to a fresh Protein LoBind tube. Do not carry magnetic beads or residual Wash Buffer into the recovered lysate.
7	**QUANTIFY OR NORMALIZE** Measure protein using a method compatible with the selected detergent and buffer, or normalize by starting sample input when appropriate for the validated assay.
8	**USE OR STORE** Proceed immediately to the downstream workflow or freeze the cleared lysate at −80°C. Avoid repeated freeze-thaw cycles.

FOR DEEP LC-MS/MS Use an MS-compatible cleanup and digestion workflow. For maximal proteome depth and mass-spectrometry-focused studies, use the dedicated EVs360™ Proteome-MS Kit (Cat. no. L20001 · 10 rxn or L20002 · 25 rxn).

Downstream guidance

- Western blotting / ELISA: verify that the selected lysis buffer is compatible with antibodies, standards, and detection chemistry.
- Olink® / NuLISA™ / multiplex assays: follow the platform input and buffer requirements; dilution or buffer exchange may be necessary.

Completion checklist

- All three EV-capture washes were completed and residual Wash Buffer was removed.
- The bead pellet was fully resuspended during each vortex cycle.
- Three vortex-ice cycles were completed.
- Magnetic beads remained behind during lysate transfer.
- The lysis buffer, inhibitor use, protein measurement, and storage location were documented.