EVs360™ Proteome-MS Kit

Operator Manual for LC–MS/MS and Deep Proteomics

PROTEOMICS-READY ENRICHMENT Rapid, marker-independent extracellular-vesicle capture optimized for LC–MS/MS, deep proteomics, and high-sensitivity protein analysis.

Product configuration	Validated input per reaction
EVs360™ Proteome-MS Kit, Cat. no. L20001 · 10 rxn	0.25 mL plasma/serum or 2.5 mL cell culture media
EVs360™ Proteome-MS Kit, Cat. no. L20002 · 25 rxn	0.25 mL plasma/serum or 2.5 mL cell culture media

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Start here

A proteomics-focused overview for a consistent first run.

What the kit does

Lipid nanoprobe- magnetic beads capture extracellular vesicles through vesicular membranes, enabling unbiased pan-EV enrichment without a surface-marker requirement. The workflow is optimized for in-depth LC–MS/MS, deep proteomics, and other high-sensitivity protein platforms.

Designed for protein discovery

- 15-minute EV capture without ultracentrifugation.
- Low-input plasma/serum and cell-culture workflows.
- Three controlled washes to reduce soluble-protein background.
- Protein LoBind handling to minimize sample loss.
- Compatible with LC–MS/MS, Olink®, NuLISA™, ELISA, Western blotting, targeted biomarker validation, phosphoproteomics, and glycoproteomics.

Before you begin: operator checklist

- Confirm the sample type and validated input on page 4.
- Use Protein LoBind tubes from EV capture through lysis.
- Select an MS-compatible lysis/digestion workflow before starting.
- Prepare a magnetic rack, rotator, vortex mixer, microcentrifuge, timer, and ice.
- Resuspend Magnetic Beads L2 thoroughly before pipetting.

> **CRITICAL LC–MS DECISION** Detergent-containing buffers may require cleanup before mass spectrometry. Confirm compatibility with the downstream digestion and cleanup workflow.

Kit contents and proteomics setup

Component	L20001 · 10 rxn	L20002 · 25 rxn	Storage
Magnetic Beads L2	25 mg (5 mL)	62.5 mg (12.5 mL)	2–8°C
Solution A	5 mL	12.5 mL	Room temperature
Wash Buffer	12 mL	30 mL	Room temperature

Each reaction is designed for 0.25 mL plasma/serum or 2.5 mL cell culture media. Scale proportionally only after user optimization.

Materials and equipment you supply

- Protein LoBind tubes: 1.5 mL, 5 mL, and 0.5 mL as required by the workflow.
- Vortex mixer and microcentrifuge.
- Magnetic stand compatible with the selected tube size.
- HulaMixer™ or equivalent rotator/mixer.
- Timer, ice, calibrated pipettes, and low-retention tips.

Recommended lysis options

Option	Composition	Use consideration
1× RIPA	0.05 M Tris-HCl, pH 7.4; 0.15 M NaCl; 0.25% deoxycholic acid; 1% NP-40; 1 mM EDTA	Validated for protein extraction; detergent cleanup may be required before MS.
SDS buffer	5% SDS; 10 mM triethylammonium bicarbonate (TEAB)	Use with an MS-compatible cleanup/digestion workflow.

STORAGE The kit ships at room temperature. On receipt, store each component according to its label. Do not mix reagents between kit lots.

Prepare the sample and choose the input

Plasma or serum

1	**Thaw** Thaw at 37°C for 5–10 minutes or overnight at 4°C until completely thawed.
2	**Homogenize** Invert gently to homogenize the sample.
3	**Optional pre-clear** Centrifuge at 500 × g for 5 minutes and transfer the supernatant.

Cell culture media

1	**Remove cells** Centrifuge at 300 × g for 10 minutes.
2	**Remove debris** Transfer the supernatant and centrifuge at 2,000 × g for 10 minutes.
3	**Optional small-EV enrichment** Centrifuge at 10,000 × g for 20 minutes or filter through 0.22 µm to reduce larger microvesicles.
4	**Hold cold** Keep the prepared sample on ice.

Validated reaction setup

Sample	Input	Magnetic Beads L2	Solution A	Starting tube
Plasma / serum	0.25 mL	0.5 mL (2.5 mg)	0.5 mL	1.5 mL Protein LoBind
Cell culture media	2.5 mL	0.5 mL (2.5 mg)	Skip	5 mL Protein LoBind

SAMPLE BRANCH Use Solution A only for plasma/serum. Add cell culture media directly to buffer-free beads.

Capture EVs from plasma or serum

Standard reaction: 0.25 mL sample. Scale reagents proportionally only after optimization.

1	**Resuspend** Vortex Magnetic Beads L2 thoroughly.
2	**Aliquot** Pipette 0.5 mL beads (2.5 mg) into a 1.5 mL Protein LoBind tube.
3	**Magnetize** Place on the magnetic rack for 15–30 seconds; remove storage buffer completely.
4	**Condition beads** Add 500 µL Solution A. Invert 5–10 times, then vortex for 30 seconds until homogeneous.
5	**Add sample** Add 250 µL plasma or serum.
6	**Mix** Invert gently 5–10 times.
7	**Capture** Rotate at medium speed for 15 minutes.
8	**Collect beads** Place on the magnetic stand for more than 30 seconds and carefully remove the supernatant.

PROTECT THE PELLET Keep the tube on the magnetic stand during aspiration. Avoid touching or removing the EV-bound bead pellet.

HulaMixer™ starting settings

Motion	First line	Second line
Orbital	6 rpm	4 rpm
Reciprocal	79°	7°
Vibro / Pause	3°	5

Capture EVs from cell culture media

Standard reaction: 2.5 mL prepared medium. Solution A is not used.

1	**Resuspend** Vortex Magnetic Beads L2 thoroughly.
2	**Aliquot** Pipette 0.5 mL beads (2.5 mg) into a 5 mL Protein LoBind tube.
3	**Magnetize** Place on the magnetic rack for 15–30 seconds; remove storage buffer completely.
4	**Add sample** Add 2.5 mL prepared cell culture medium directly to the buffer-free beads.
5	**Mix** Invert gently 5–10 times.
6	**Capture** Rotate at medium speed for 15 minutes.
7	**Collect beads** Place on the magnetic stand for more than 30 seconds and carefully remove the supernatant.

NEXT STEP Continue immediately to the common three-wash and lysis workflow on page 7.

Scaling note

The validated workflow uses 2.5 mL medium and 2.5 mg beads per reaction. Larger inputs may improve yield, but bead and reagent volumes should be scaled proportionally and validated for the specific matrix.

Wash, lyse, and collect protein

Use this common workflow after EV capture from either plasma/serum or cell culture media.

1	**First wash** Add 400 µL Wash Buffer and resuspend completely. For plasma/serum, mix by hand 1–2 times, vortex 10–20 seconds, spin down droplets if needed, then transfer the suspension to a 0.5 mL Protein LoBind tube. For cell culture media, transfer the bead suspension to a new 0.5 mL tube during this wash.
2	**Remove wash** Place on the magnetic stand and remove the supernatant.
3	**Wash two more times** Repeat the 400 µL wash twice, for three washes total. After the third wash, remove all Wash Buffer completely.
4	**Add lysis buffer** Add 100 µL of the selected lysis buffer.
5	**Vortex–ice cycle** Vortex vigorously for 1 minute, then place on ice for 5 minutes. Repeat the vortex–ice cycle two more times; total incubation is approximately 20 minutes.
6	**Collect protein lysate** Place on the magnetic stand and transfer the protein solution to a fresh Protein LoBind tube.
7	**Analyze or store** Proceed immediately to downstream analysis or freeze the lysate at −80°C.

LC–MS CHECKPOINT Keep the final lysate free of magnetic beads and residual Wash Buffer. Apply the planned MS-compatible cleanup/digestion workflow before LC–MS/MS.

Proteomics quality and troubleshooting

Observation	Likely cause	Operator action
Low protein yield	Sample loss or low EV abundance	Use Protein LoBind consumables, confirm complete bead resuspension, and consider a validated increase in sample input.
High background	Residual soluble plasma proteins	Confirm all three washes and complete Wash Buffer removal after the third wash.
Poor MS compatibility	Detergent carried into digestion	Use an MS-compatible cleanup/digestion method matched to the selected lysis buffer.
Inconsistent recovery	Insufficient mixing or bead loss	Use consistent vortex/rotation settings and aspirate only while the tube is on the magnet.
Beads in lysate	Incomplete magnetic separation	Return the tube to the magnetic stand and transfer the cleared lysate to a fresh tube.

Study consistency checklist

- Use the same sample preparation across comparison groups.
- Keep capture time, mixing settings, wash count, lysis buffer, and vortex–ice cycles consistent.
- Document sample input, bead amount, tube type, wash volumes, lysis buffer, cleanup method, and storage time.
- Avoid repeated freeze–thaw cycles of samples and lysates.

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