EVs360™ Proteome-MS Kit

ONE-PAGE QUICK GUIDE | LC–MS/MS BENCH WORKFLOW

1 Choose the setup

PRODUCT OPTIONS L20001 · 10 rxn | L20002 · 25 rxn

Validated reaction setup

Sample	Input	Beads L2	Solution A
Plasma / serum	0.25 mL	0.5 mL (2.5 mg)	0.5 mL
Cell culture	2.5 mL	0.5 mL (2.5 mg)	Skip

CRITICAL BRANCH Use Solution A only for plasma/serum. Skip it for cell culture media.

Prepare the sample

- Use Protein LoBind tubes from capture through lysis.
- Plasma/serum: thaw 5–10 min at 37°C or overnight at 4°C; invert gently. Optional: 500 × g, 5 min; use supernatant.
- Cell culture: 300 × g, 10 min; then 2,000 × g, 10 min. Optional: 10,000 × g, 20 min or 0.22 µm filtration. Keep on ice.

Kit storage

- Magnetic Beads L2: 2–8°C. Solution A and Wash Buffer: room temperature.

2 Capture EVs

1 PREP BEADS
- Vortex thoroughly. Aliquot 0.5 mL (2.5 mg). Magnetize 15–30 sec and remove storage buffer completely.

2 CONDITION
- Plasma/serum only: add 500 µL Solution A; invert 5–10× and vortex 30 sec. Cell culture: skip.

3 ADD SAMPLE
- Add 250 µL plasma/serum to conditioned beads, or 2.5 mL cell culture media to buffer-free beads. Invert 5–10×.

4 CAPTURE
- Rotate at medium speed for 15 min.

5 COLLECT
- Magnetize for >30 sec. Remove supernatant without disturbing the beads.

PROTECT THE PELLET Keep the tube on the magnet during aspiration.

3 Wash three times

1 FIRST WASH
- Add 400 µL Wash Buffer; resuspend completely. Transfer to a 0.5 mL Protein LoBind tube.

2 REMOVE WASH
- Magnetize and remove the supernatant.

3 REPEAT TWICE
- Perform two more 400 µL washes. After wash 3, remove all Wash Buffer.

PROTEOMICS CHECKPOINT Three consistent washes and complete final aspiration help reduce soluble-protein and buffer carryover.

Recommended lysis options

Option	Composition / note
1× RIPA	0.05 M Tris-HCl pH 7.4; 0.15 M NaCl; 0.25% deoxycholic acid; 1% NP-40; 1 mM EDTA.
SDS	5% SDS; 10 mM TEAB. Use an MS-compatible cleanup/digestion workflow.
Urea	8 M urea and 0.1 M ammonium bicarbonate

4 Lyse and collect protein

1 ADD BUFFER
- Add 100 µL of the selected lysis buffer.

2 VORTEX–ICE
- Vortex 1 min; ice 5 min. Repeat two more times (total incubation ~20 min).

3 CLEAR LYSATE
- Place on the magnet and transfer lysate to a fresh Protein LoBind tube.

4 ANALYZE / STORE
- Proceed immediately or freeze at −80°C.