ATLAS · EV ELISA

How should I quantify my EVs from biofluids? Western blot, ELISA, or single-particle counting

Atlas™ EV ELISA is for research use only. Not for use in diagnostic procedures.

The need for protein markers to distinguish particles from EVs

Accurate measurement of extracellular vesicles (EVs) in biofluids is essential for optimizing isolation workflows, biomarker discovery, and translational research. Label-free single-particle methods: flow cytometry, NTA, and TRPS/MRPS — are the go-to tools for quantifying EVs. They detect particles within a defined size range but cannot distinguish vesicles from other nano-sized components common in biological fluids.

In complex biofluids such as plasma or serum, lipoproteins and protein aggregates overlap with EVs in both size and density, complicating accurate measurement. MISEV2023 now formally distinguishes "particle concentration" from "EV concentration" and recommends orthogonal, protein-based methods to close this gap.

Specificity can be improved by detecting EV-associated proteins such as CD9, CD81, and CD63 through fluorescent labeling. However, fluorescent labeling for single-particle analysis requires optimization, extensive controls, and is limited by sensitivity.

ELISA VS. WESTERN BLOT

Traditional ELISAs are designed to detect soluble proteins and so lack EV specificity, but the format's sensitivity and throughput make it a strong foundation. The table below compares the two methods across parameters relevant to EV protein analysis.

Parameter	ELISA	Western Blot
Detection principle	Antibody capture + enzymatic signal amplification	Electrophoresis + antibody detection
Detection context	Native proteins	Denatured proteins after SDS-PAGE
Analytical sensitivity	High–moderate (readout-dependent)	Low
Dynamic range	2–4 logs (readout-dependent)	Limited
Specificity	Antibody-pair dependent	Antibody binding with molecular-weight confirmation
Performance in complex biofluids	Compatible with plasma, CSF, urine, saliva, conditioned media	Reduced in plasma due to high protein background
Time to result	Same-day (~hours)	~1.5–2 days
Workflow complexity	Simple; 1–3 incubation steps + wash	Multi-step (electrophoresis, transfer, antibody incubations)
Throughput / scalability	High; plate-based, automation-compatible	Low; manual, lane-based workflow

Western blot: orthogonal confirmation with broad limitations

Western blot, an inherently multi-step, laborious method, has traditionally confirmed canonical EV markers such as CD9, CD63, and CD81. By separating proteins by molecular weight, it adds a layer of molecular-identity verification.

Because it analyzes denatured protein, however, Western blot cannot distinguish EV-associated markers from non-vesicular forms, a critical limitation when free-protein contamination is high.

Performance also declines in complex samples such as plasma, producing smeared bands that are difficult to resolve, and its limited sensitivity prevents detection of low-abundance proteins.

ELISA-based immunoassays address these workflow and sensitivity limitations. With same-day readouts and plate-based throughput, ELISA enables reproducible, efficient EV analysis across complex biofluids.

Combining Western blot and ELISA for complementary analysis

In practice, the two are most effective together. Ter-Ovanesyan et al., optimizing immuno-isolation of neuron-specific plasma EVs, used ELISA to measure tetraspanin depletion in the supernatant while Western blot produced only a smear. Western blot confirmed target enrichment in the pulldown, which ELISA cannot quantify, since measuring bead-bound antigen requires disrupting the antigen–antibody interaction to elute the target. Together, Western blot confirms marker presence and ELISA confirms its depletion from the supernatant, enabling EV-subtype pull-down optimization.

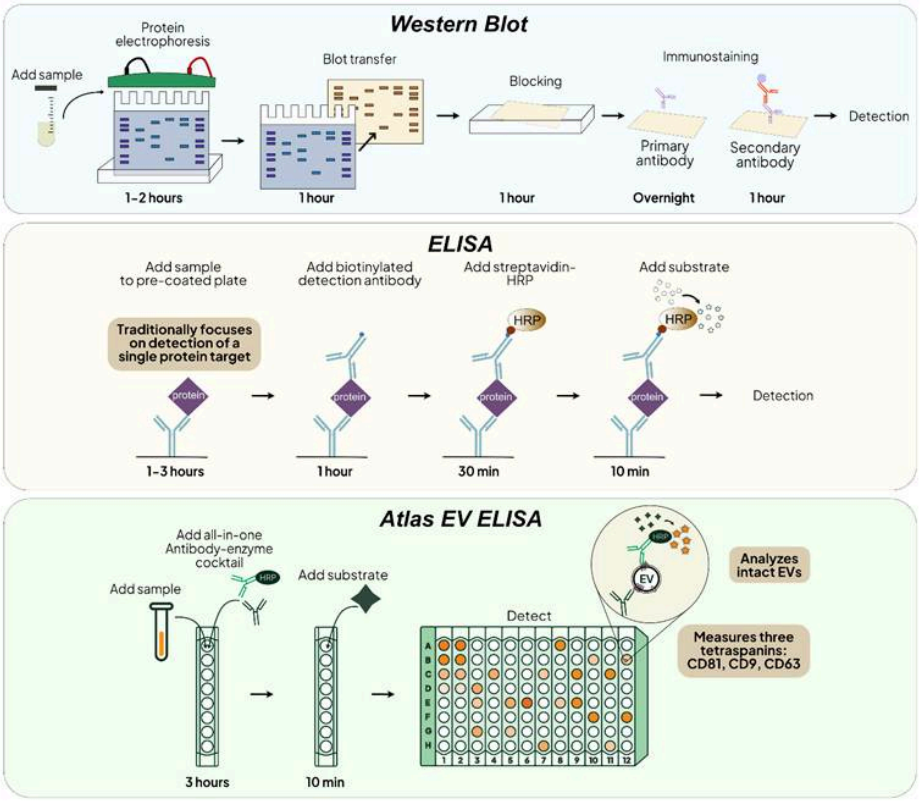

Figure 1. Workflow schematics for protein detection by Western blot, conventional ELISA, and the intact-EV Atlas EV ELISA.

Atlas EV ELISA: a multi-target, intact-EV assay

To overcome the limits of traditional ELISA for EV quantification, Everest Biolabs developed the Atlas EV ELISA, an intact-EV assay that simultaneously targets the three most abundant tetraspanins: CD81, CD63, and CD9. The plate-based format quantifies EV-associated proteins and evaluates sample purity using only 50 µL of biofluid or SEC fraction, with ~15 minutes of hands-on preparation.

Atlas absorbance ELISAs are especially powerful for quantifying EV yield and purity across SEC fractions, fine-tuning fraction collection for downstream applications. The Atlas Lumi Human EV ELISA adds a chemiluminescence readout for the higher sensitivity and wider dynamic range needed for low-abundance samples such as CSF or dilute culture media, and the Atlas Lumi Homebrew offers a customizable format for markers beyond the tetraspanins.

SUMMARY

Label-free single-particle detection techniques provide the total particle concentration and are not EV-specific. Western blot is widely used to confirm the presence of canonical EV markers such as CD9, CD63, and CD81 through orthogonal affinity- and molecular weight-based detection, but it provides limited quantitative or surface-specific insight into EV populations. In contrast, Atlas EV ELISA measurement of surface markers on intact vesicles enables scalable, quantitative, and biologically interpretable assessment of EV abundance and variation across biofluids and clinical states. Together, these distinctions position Western blot primarily as a confirmatory identity tool, while EV ELISA serves as a more suitable platform for sensitive, reproducible, and cohort-scale EV biomarker measurement.

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