ATLAS · EV ELISA

Atlas EV ELISA: a scalable solution for extracellular vesicle quantification

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

Accurate quantification of extracellular vesicles (EVs) underpins reproducible EV research, biomarker discovery, and therapeutic development. Label-free single-particle methods report total particle concentration but cannot distinguish EVs from similarly sized lipoproteins and protein aggregates, especially abundant in plasma and serum.[1,2]

The Atlas EV ELISA is a plate-based immunoassay that quantifies intact EVs by simultaneously targeting the three most abundant tetraspanins: CD9, CD63, and CD81. Requiring only 50 µL of biofluid or SEC fraction and ~15 minutes of hands-on time, it delivers EV-specific, quantitative, scalable measurements suited to fraction optimization, cohort studies, and cross-sample standardization.

How the Atlas EV ELISA works

The assay captures intact vesicles on a plate coated with anti-tetraspanin antibodies and detects them with a second tetraspanin antibody, generating a signal proportional to EV abundance. Because both capture and detection rely on EV surface markers, the readout reflects genuine vesicles rather than total particles. Two formats are available:

- **Atlas Absorbance EV ELISA**: colorimetric readout for routine, cost-effective quantification across most biofluids and SEC fractions.
- **Atlas Lumi EV ELISA**: chemiluminescent readout with higher sensitivity and wider dynamic range for low-abundance samples such as CSF or dilute culture media.

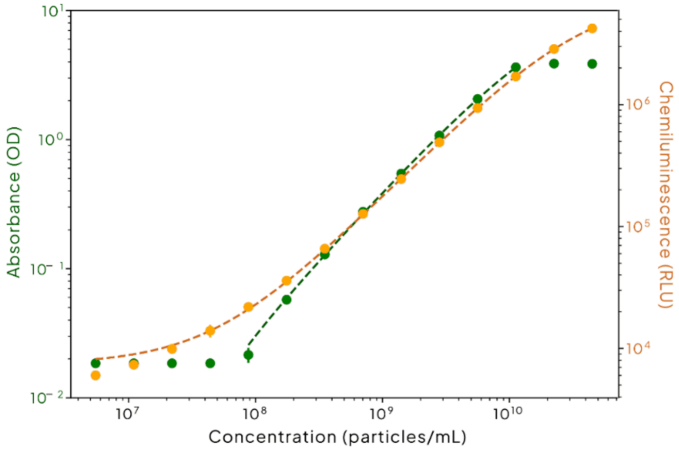


Figure 1. Atlas EV ELISA standard curves. Signal versus EV concentration for the Absorbance and Lumi formats. The chemiluminescent Lumi format extends sensitivity to lower EV concentrations (LOD ~10^7 particles/mL) and offers a wider dynamic range than the colorimetric Absorbance format (LOD ~10^8 particles/mL).

ASSAY WORKFLOW

1 Add sample
50 µL sample + detection antibody into coated wells

2 Incubate
Intact EVs captured via tetraspanins & bound by detector

3 Wash
Remove unbound material to minimize background

4 Add substrate
Colorimetric (Absorbance) or chemiluminescent (Lumi)

5 Read & quantify
Signal vs. standard curve gives EV concentration

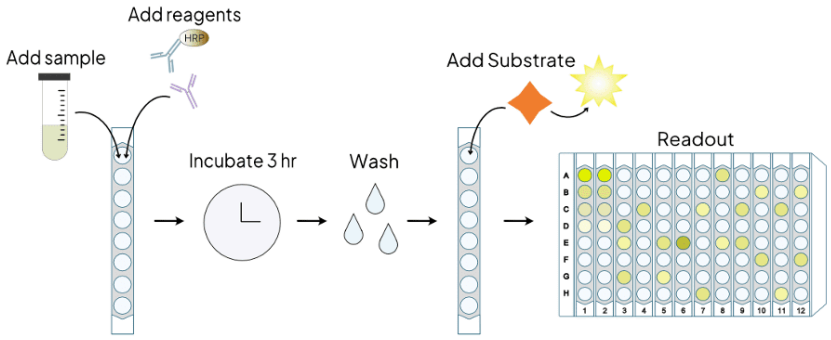


Figure 2. Assay workflow. Intact EVs are captured and detected through their surface tetraspanins (CD9, CD63, CD81), producing a colorimetric or chemiluminescent signal proportional to EV abundance.

APPLICATIONS: SEC FRACTION OPTIMIZATION

Because it is fast and uses minimal sample, the Atlas Absorbance EV ELISA is well suited to mapping EV elution across SEC fractions. Quantifying EV signal, alongside co-eluting protein, fraction by fraction lets researchers identify the fractions of highest EV yield and purity, then tailor pooling and collection for downstream applications such as proteomics or functional studies (Figure 3).

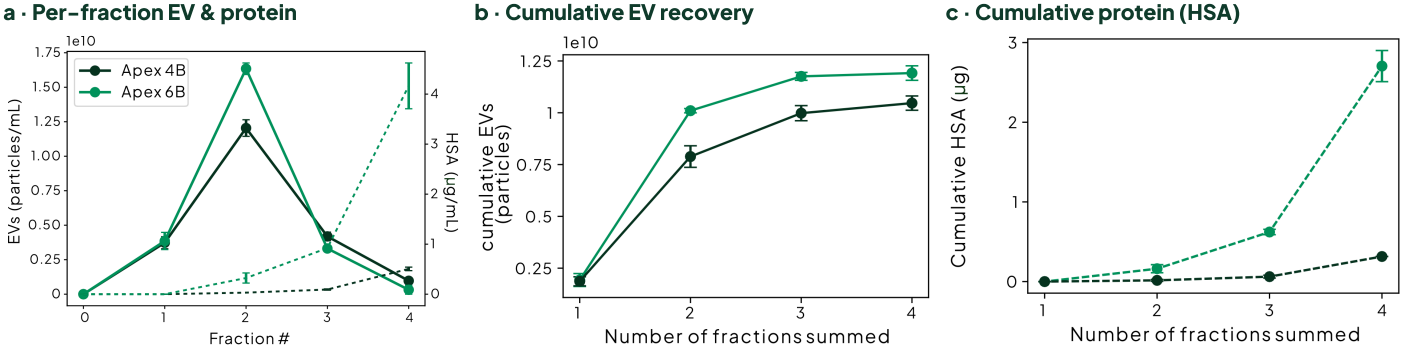


Figure 3. Fraction-resolved EV quantification. (a) Atlas EV ELISA EV signal and HSA (protein) across SEC fractions identify the EV-enriched peak and separate it from later-eluting soluble protein. (b, c) Cumulative EV recovery and cumulative protein carryover as successive fractions are pooled, guiding fraction pooling for optimal yield and purity.

Comparison to single-particle analysis

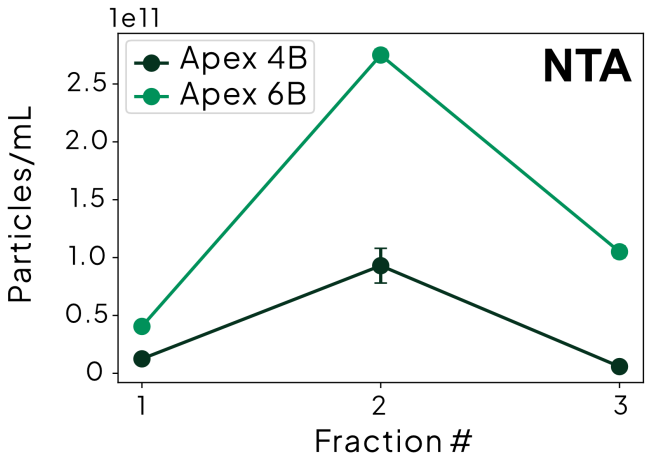
On a purified EV standard, the Atlas EV ELISA reports EV concentrations broadly consistent with orthogonal single-particle platforms: flow cytometry, NanoFCM, and NTA. In complex samples such as plasma, however, label-free single-particle methods substantially overestimate EV concentration because they also count co-isolating lipoproteins and aggregates. Being tetraspanin-specific, the Atlas EV ELISA is not inflated by these non-vesicular particles, giving a more biologically meaningful measure of EV abundance (Table 1).

Sample	Atlas EV ELISA	Flow cytometry	NanoFCM	NTA
Purified EV standard	5.0×10^{10}	2.9×10^{10}	1.4×10^{10}	2.9×10^{10}
Plasma EVs	7.9×10^{10}	2.7×10^{10}	1.9×10^{10}	5.7×10^{11}

Table 1. *EV concentration (particles/mL) by the Atlas EV ELISA and three single-particle platforms. Methods agree on a purified standard; in plasma, label-free NTA markedly overestimates concentration owing to non-vesicular particles.*

Across SEC fractions, Atlas EV ELISA and NTA show similar EV elution profiles; however, NTA consistently reports particle counts five- to ten-fold higher than the Atlas EV ELISA, especially with the Apex 6B column, which co-elutes small lipoproteins, confirming that the ELISA more accurately reflects true EV content rather than total particle counts (Figure 4).

NTA



Atlas EV ELISA

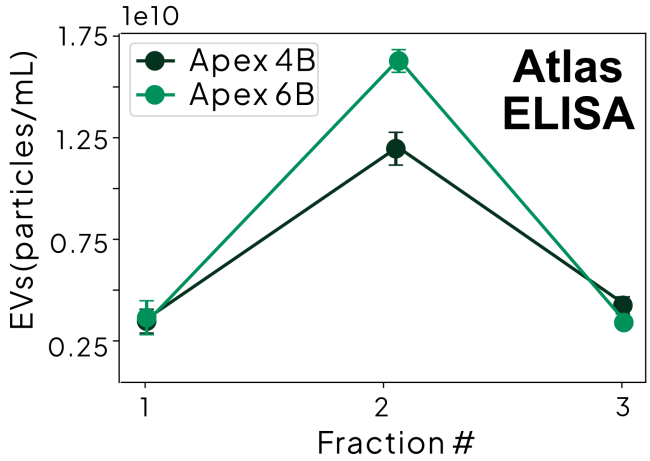


Figure 4. NTA vs. Atlas EV ELISA across SEC fractions. Both methods show a similar EV elution profile, but NTA (left) reports particle counts roughly five- to ten-fold higher than the Atlas EV ELISA (right), especially on the Apex 6B column, because NTA also counts non-EV particles such as co-eluting lipoproteins.

Atlas Lumi ELISA with the Homebrew conjugation kit

For markers beyond the core tetraspanins, the Atlas Lumi Homebrew kit lets researchers conjugate their own antibodies into the chemiluminescent format, extending EV-specific, high-sensitivity quantification to custom targets, such as CD41 for platelet-derived EVs (Figure 5).

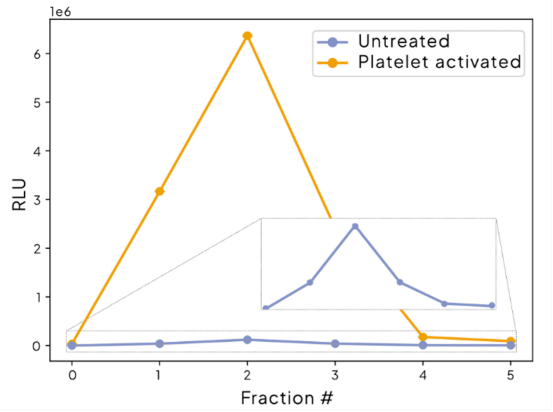


Figure 5. Custom-marker detection with the Atlas Lumi Homebrew kit. A researcher-conjugated antibody (e.g. CD41) is used in the chemiluminescent format to quantify a specific EV subpopulation.

SUMMARY

- **Two formats**: colorimetric (Absorbance) and chemiluminescent (Lumi) — spanning routine and low-abundance applications.
- **Minimal input**: 50 µL of biofluid or SEC fraction with ~15 minutes of hands-on time.
- **EV-specific**: targets CD9, CD63, and CD81 on intact vesicles, avoiding lipoprotein-driven overestimation.
- **Scalable**: plate-based and automation-compatible for fraction optimization and cohort studies.

Explore the Atlas EV ELISA kits →

REFERENCES

1. Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023). *J Extracell Vesicles*. 2024.
2. Hartjes TA, Mytnyk S, Jenster GW, et al. EV quantification and characterization: common methods and emerging approaches. *Bioengineering (Basel)*. 2019.
3. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *J Extracell Vesicles*. 2018.
4. Ter-Ovanesyan D, Norman M, Lazarovits R, et al. Framework for rapid comparison of EV isolation methods. *eLife*. 2021.