Atlas

Human EV ELISA Quick Guide

Revision 3 – May 7, 2025

The Atlas Human EV ELISA is an immunoassay to measure intact extracellular vesicles (EVs) in plasma, serum, urine, cell culture media, or cerebrospinal fluid (CSF). This kit contains enough reagents for 96 wells.

Supplied kit contents:

Item	Quantity	Storage
Human EV Capture Antibody 100X	80 µL	4° C
Human EV Detection Antibody 100X	80 µL	4° C
Diluent	50 mL	4° C
Wash Buffer 10X	25 mL	4° C or Room temperature (RT)
TMB Solution	12 mL	4° C
Stop Solution	12 mL	4° C or RT
Pre-Coated 96-Well Microplate	96-well	4° C
Plate Seals	3	RT
EV standard	1 tube	4° C or -20° C

Materials needed:

- Microplate reader (450 nm absorbance)
- Plate Shaker
- Low-bind Eppendorf tubes
- Lab-grade deionized water
- 8 channel pipettor (300 µL) or a plate washer (recommended)

About the kit:

- The kit provides enough reagents for 96 wells (12 x 8 well strips).
- Capture and detection antibodies have only been tested for stability in the 100X formulations.
- **This assay is for research use only, not for diagnostic procedures.**

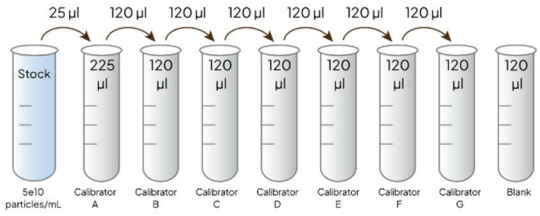
Reagent preparation:

- Equilibrate reagents to room temperature (18-25°C) before use.
- Briefly spin down capture and detection antibody stocks.
- The volumes below are sufficient for one strip (8 wells); adjust volumes as needed.
- Prepare only the required volume of reagents for each experiment.

1X Wash Buffer:
Prepare 1X Wash Buffer by diluting Wash Buffer 10X with deionized water.
To make 25 mL of 1X Wash Buffer, combine 2.5 mL Wash Buffer 10X with 22.5 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail:
Prepare Antibody Cocktail by diluting the capture and detector antibodies in Diluent.
To make 500 µL of the Antibody Cocktail, combine 5 µL of Capture Antibody 100X and 5 µL of Detector Antibody 100X with 490 µL of Diluent. Mix thoroughly and gently.

EV Standard:
Reconstitute the EV standard by adding the volume of diluent indicated on the label to obtain a stock concentration of 5×10^{10} particles/mL. Aliquot stock and freeze for future experiments. Dilute the stock 10-fold to prepare a 5×10^{9} particles/mL standard (Calibrator A). We recommend preparing 2-fold serial dilutions from Calibrator A.



*** Particle concentration was calculated based on the average number of tetraspanins per EV in biofluids**.

Item	Concentration (particles / mL)	Preparation
EV Standard	5×10^{10}	Resuspend in volume of Diluent on EV Standard label
Calibrator A	5×10^{9}	25 µL of EV Standard to 225 µL of Diluent
Calibrator B	2.5×10^{9}	120 µL of Calibrator A to 120 µL of Diluent
Calibrator C	1.25×10^{9}	120 µL of Calibrator B to 120 µL of Diluent
Calibrator D	6.25×10^{8}	120 µL of Calibrator C to 120 µL of Diluent
Calibrator E	3.125×10^{8}	120 µL of Calibrator D to 120 µL of Diluent
Calibrator F	1.5625×10^{8}	120 µL of Calibrator E to 120 µL of Diluent
Calibrator G	7.8125×10^{7}	120 µL of Calibrator F to 120 µL of Diluent
Blank	0	120 µL of Diluent

Sample preparation:
Prepare 75 µL of each sample to be tested. Dilute samples at least 2X using Diluent.

Note: Spin samples at 2,500 g for 10 minutes to remove platelets and debris.

Typical Sample Dynamic Range	
Assay Range (O.D)	0.03 – 1.90
Sample Type	**Dilution Range**
Plasma	4X – 64X
Serum	4X – 64X
Urine	2X – 8X
CSF	2X – 32X
Culture Media	2X – 32X

Assay procedure:

1. Prepare all reagents, standards, and samples as directed above.
2. Remove excess microplate strips from the plate frame.
   - Seal and return them to 4° C storage.
   - The remaining strips should be used within two weeks if kept sealed.
3. Add 50 µL of each sample or standard to separate wells in the microplate strips.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 3 hours at room temperature on a plate shaker (400 rpm) or overnight at 4°C. Protect the plate from light during incubation.
6. Wash each well five times:
   - For the first wash, add 200 µL 1X Wash Buffer to the wells containing the samples.
   - Aspirate each well.
   - Dispense 350 µL 1X Wash Buffer. The wash buffer should remain in the wells for at least 10 seconds.
   - Aspirate each well. Ensure complete removal of liquid between washes.
   - Repeat four times for a total of 5 washes.
   - After the final wash, blot the plate against clean paper towels.

To prevent cross-contamination, blot the plate only after the final wash.
For consistent results across replicates, ensure wells are completely emptied between each wash.

7. Add 100 µL of TMB Substrate Solution to each well. Incubate for 10 minutes in the dark on a plate shaker at 400 rpm.
8. Add 100 µL of Stop Solution to each well. Shake for 1 minute. The solution will change from blue to yellow.
9. Measure the absorbance at 450 nm (endpoint reading). If wavelength correction is available, set to 620 nm.

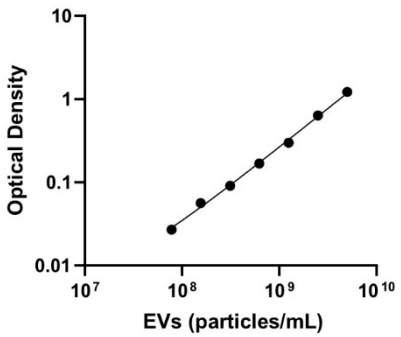
Data analysis:

We recommend running the samples and calibrators in duplicate. To calculate the results, average the duplicate OD readings and subtract the average OD of the zero standard. Fit the standard curve using 4PL or a linear regression on a log-log plot. Interpolate the standard curve to determine the samples' concentrations. Multiply the calculated concentration by the dilution factor to obtain the final concentration. A new standard curve should be generated with every experiment.

Warnings and precautions:

The Diluent contains ProClin 300®, which may cause an allergic skin reaction. Avoid ingestion and contact with eyes, skin, and mucous membranes.

Example of a standard curve:



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