Abbelight
NOW, WE SEE

Abbelight™ Smart EVs Kit

A ready to use kit to capture, label and visualize your EVs in SMLM

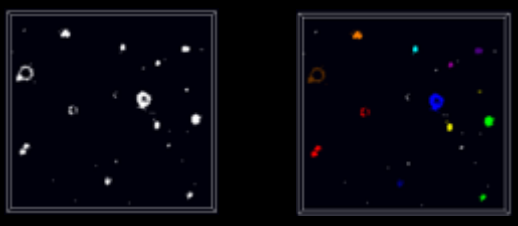
Extracellular vesicles (EVs) are membrane bound particles, with nanometer-range size (20-5000 nm) that are secreted by all cell types, and are currently recognized as important actors in many physiological and pathological processes.[1-4] The biological features and properties of secreted EVs reflect cell behavior and/or its pathophysiological status, such as cell growth, migration, cleavage, and death. Moreover, cells increase the production of EVs under pathological conditions such as infection[5] or cancer[6,7], whereby they sustain and modulate the disease progression. Thus, EVs offer unprecedented perspectives for personalized diagnosis and therapy[8].

EVs' specific biological role and their effect upon cellular uptake highly depends on their biogenesis process, their surface proteins, and their cargo. In particular, the content of the EVs includes several bioactive compounds, such as proteins, enzymes, lipids and nucleic acids, including microRNAs and mRNAs, that are actively sorted within the EVs .

In compliance with MISEV 2018 guidelines[9], a thorough characterization of the structural and biochemical features of the EVs is thus essential, especially in the medical and pharmaceutical fields. Classical optical microscopy lacks the resolution to dissect small vesicles whose size can be well below the diffraction limit.

Single molecule localization microscopy (SMLM) offers the highest spatial resolution among super-resolution techniques (10-20 nm in x-y axis), achieving true molecular resolution. By separating in time the emission of single fluorophores, it is possible to reconstruct their spatial position with a very high precision, generating spatial coordinate tables that can be then analyzed with Abbelight's data analysis software NEO. More in particular, by using clustering algorithms, each single EV (or cluster) can be separated and analyzed singularly.

Clustering



abbelight.com

Abbelight™ Smart EVs Kit

To optimally perform SMLM to image and characterize EVs, it is necessary to immobilize them onto a surface. The available protocols are long and require optimization steps that can be tedious.
To facilitate the study of EVs, Abbelight has developed for its customers a ready-to-use kit to capture, label and image your EVs. This kit is stable, tunable, and easy to use to fit all your experiment needs.

Product description

The EVs kit allows you to capture, label and visualize EVs in specific sub-populations in a simple and fast way while ensuring high stability and repeatability of your experiments. Its tunability allows to fit many applications, especially approaching SMLM imaging in the best possible conditions.

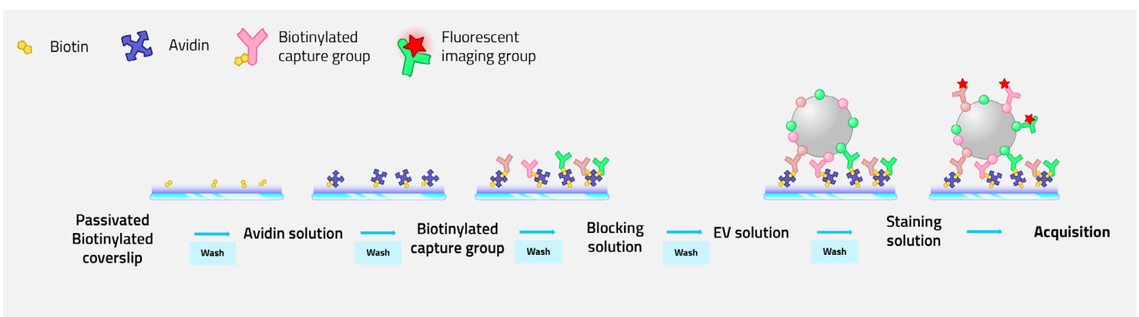
Kit components:

- Biotinylated glass slides
- Microfluidic chambers
- Washing buffer
- Capture solution
- Biotinylated antibodies solution
- Blocking solution
- Labeling solution
- Control EVs provided by EVerZom
- Smart kit buffer

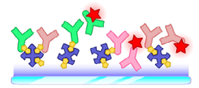
Features and benefices

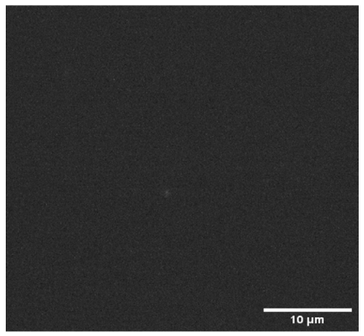
- **Ready-made solutions** - Optimized and ready-to-use solutions to easily capture all your Evs in an efficient way
- **Robust and reliable sample preparation** - This kit has been designed to assure the highest level of reproducibility. The solutions included in the kit have a strictly controlled formulation and have been optimized to yield the best results in fluorescence imaging, with inter-assay variability <15%.
- **Well known and validated capture groups** - The kit offers you the best combination of capture groups without having to spend hours finding the right experimental parameters, thus saving you precious time
- **Customizable** - add your own biotinylated antibodies to customize your kit

How it works?

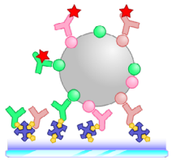


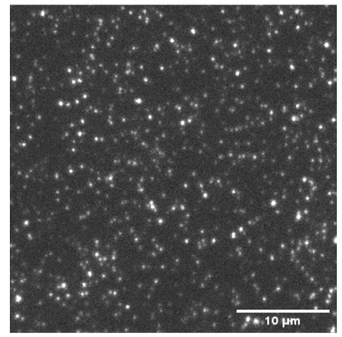
Specific and selective capture





negative control



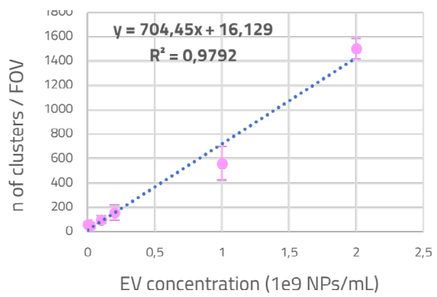


EVs (2 · 10^9 NPs / mL)

The EVs kit allows you to selectively and specifically capture the EVs from your sample. Left: fluorescence diffraction-limited image of a negative control (i.e., without EVs). Right: fluorescence diffraction-limited image of EVs from EVerZom

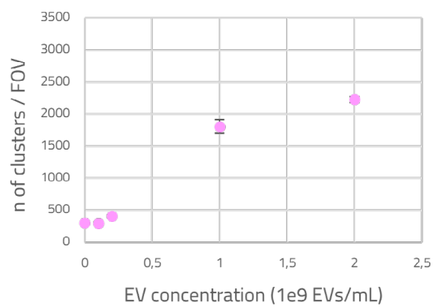
EV concentration working range

Working EV concentration (TMIX):



Anti-tetraspanins (CD9, CD63 and CD81) based capture: linear relationship between the number of EVs immobilized on an anti-tetraspanins surface per field of view and concentration of EV sample. (Field of view: 40x40 µm)

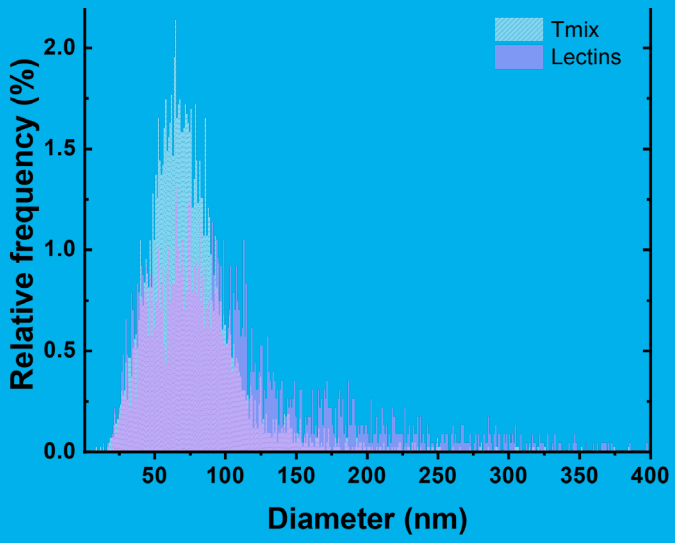
Working EV concentration (lectins):



Lectin-based capture: Logarithmic relationship between number of EVs immobilized on a lectin surface per field of view and concentration of EV sample. (Field of view: 40x40 um)

Specific and selective capture

Comparison between our two capture strategies



Size distribution obtained by SMLM of EVs immobilized on a tetramix-based capture (black) and a lectin-based capture (pink)

Duration over time	2 months
Number of slides	5 slides with 6 wells each
Capture groups	Anti-CD9, Anti-CD63, Anti-CD81 - biotin WGA, ConA - biotin
Imaging groups	Anti-CD9, Anti-CD63, Anti-CD81– AF647
Control EVs	hASC extracellular vesicules from EVerZom

How to validate your own capture groups?

- What is the nature of the sample? Could its contaminants interfere with the efficient capture of EVs?
- Concentration of the biotinylated capture group solution incubated on the surface -> at least ~250 nM (for IgG is ~1 µg/mL)
- Construct a calibration curve with at least three EV concentrations and a negative control (three experimental replicates)
- Calculate LOD = average of clusters in negative control + 3 * standard deviation of clusters in negative control
- Define a concentration working range (between LOD and start of the plateau of the calibration curve)
- Define whether your background level is acceptable for the EV concentration you expect to measure routinely

References
1. B. Hugel, M.C. Martinez, C. Kunzelmann, J.M. Freyssinet, Physiology (Bethesda, Md.), 20 (2005) 22-27.
2. J. Ratajczak, M. Wysoczynski, F. Hayek, A. Janowska-Wieczorek, M.Z. Ratajczak, Leukemia, 20 (2006) 1487-1495.
3. G. Camussi, M.C. Deregibus, S. Bruno, V. Cantaluppi, L. Biancone, Kidney international, 78 (2010) 838-848.
4. M. Krause, A. Samoylenko, S.J. Vainio, Frontiers in cell and developmental biology, 3 (2015) 65.
5. P.S. Prakash, C.C. Caldwell, A.B. Lentsch, T.A. Pritts, B.R. Robinson, The journal of trauma and acute care surgery, 73 (2012) 401-406.
6. R. Valenti, V. Huber, M. Iero, P. Filipazzi, G. Parmiani, L. Rivoltini, Cancer research, 67 (2007) 2912 2915.
7. K. Al-Nedawi, B. Meehan, J. Micallef, V. Lhotak, L. May, A. Guha, J. Rak, Nature cell biology, 10 (2008) 619-624.
8. M. Piffoux, A. Nicolás-Boluda, V. Mulens-Arias, S. Richard, G. Rahmi, F. Gazeau, C. Wilhelm, A.K.A. Silva, Adv Drug Deliv Rev. (2019) 247-258
9. Théry, C. et al. J. Extracell. Vesicles (2018)

contact@abbelight.com

191 avenue Aristide Briand
94230 Cachan - France

abbelight.com

Follow us!

2023050031_AbbelightEVsbrochure