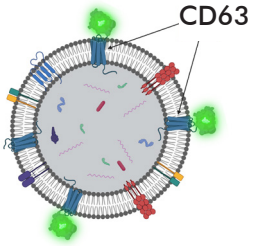
FLuoEVs: purified EVs expressing fluorescent proteins

1- About FLuoEVs:

FLuoEVs are a next generation of stably-fluorescent small EVs/ Exosomes expressing the fluorescent protein EGFP (green), BFP (blue) or mCHERRY (Red). FLuoEVs are purified by combination of Tangential Flow Filtration (TFF) and Size Exclusion Chromatography (SEC) from cells engineered in order to express the fluorescent proteins as fusion protein with the tetraspanin CD9, CD63 or CD81. Compared to the EVs labelled with fluorescent membrane dyes, FLouEVs demonstrated and increased stability of the fluorophore and a higher percentage of the fluorescent particles.

2- Types of FLuoEVs available:



- Currently available from HEK293 cells, expressing EGFP (green) as fusion protein with CD63.

3- Specifications:

Number of Fluorescent particles per vial	>1x10^9 particles
Volume prior lyophilization per vial	100 µl
Buffer prior lyophilization	PBS 1X

4- Procedure for FLuoEVs reconstitution:

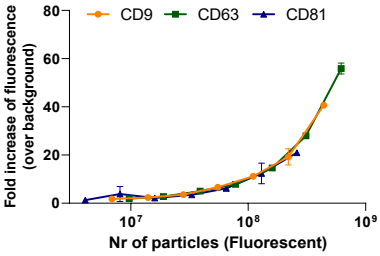
- Reconstitute FLuoEVs by adding 100 µl of deionized water (MilliQ). Resuspend the EVs by pipetting the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted EVs for 60 seconds.
- Briefly centrifuge the tubes containing the standard to ensure that the solution is collected at the bottom of the tube. Pipette the solution up and down 10 times, avoiding the introduction of bubbles. After this step, the FLuoEVs is ready to use.

5- Storage:

- FluoEVs can be stored for 12 months at 4°C, in lyophilized form.
- Reconstituted FLuoEVs are not suitable for long term conservation at room temperature; use them within 2 hours after reconstitution. The remaining reconstituted solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze-and-thaw cycles.

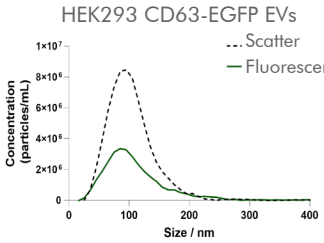
6- Application of FLuoEVs

6a- detection of FLuoEVs on plate reader.

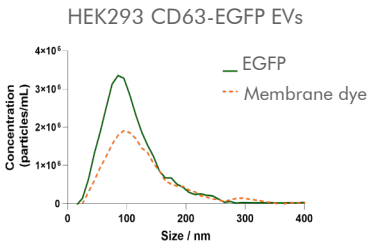


Dilution curve of FluoEVs expressing EGFP as fusion protein respectively with CD9, CD63, CD81.

6b- Nanoparticle tracking Analysis in Fluorescent mode

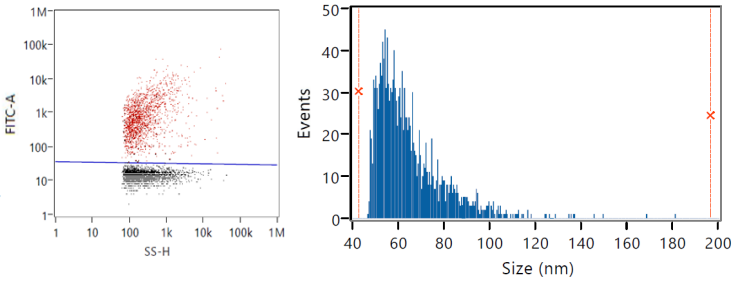


FLuoEVs HEK293-CD63-EGFP Analysis in scattered vs fluorescence mode (percentage of fluorescent partilces 40 - 60 %).



Comparison of FLuoEVs HEK293-CD63-EGFP and HEK293 EVs labeled with the lipidic dye Bodipy.
No bleaching observed compared to membrane dyes.

6c- Flow Cytometry



FLuoEVs HEK293-CD63-EGFP analysis by NanoAnalyzer NanoFCM (percentage of fluorescent partilces 60 - 80 %)