NanoSorter® Meets FLUO-EVs: Precision Sorting of Extracellular Vesicles

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Introduction

Extracellular vesicles (EVs) are nanosized membrane-bound particles released by cells and present in a wide variety of biofluids. Research on EVs have the potential of revolutionizing medicine. EVs excreted by tumor cells show great potential as noninvasive cancer biomarkers. Yet, there is currently a lack of tools to fully unlock EVs' potential in clinics. Today there is no lab equipment able to rapidly sort specific EVs subpopulations and get access to their functions and potential. This application note describes how the patented NanoSorter® technology by HEKAT Fluidics can achieve sorting of nano-objects exhibiting fluorescent tags, and how FLUO-EVs by HansaBioMed can be utilized as positive control for such technology.

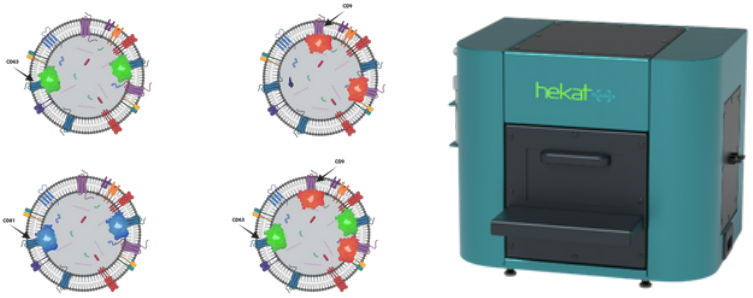

Figure 1: Representative images of FLUO-EVs and NanoSorter® benchtop version

Materials and Methods

In this study, HansaBioMed's FLUO-EVs (HBM-HEK-eGFP-63 and HBM-HEK-mCherry-9-eGFP-63) were measured. As a result of genetic modification to their parent cells, these EVs stably express fluorescent proteins in fusion with tetraspanin markers. Therefore, they present great potential as positive controls.

At reception, lyophilized EVs were stored at 4°C. On the experiment day, EVs were reconstituted using deionized water. The optical excitation-emission track setup of Nanosorter® was adapted to targeted fluorochromes (ex: 488 nm / em: 525-39 nm for eGFP; ex: 532 nm / em: 620-52 nm for mCherry). Run duration was set at 120 seconds and each measure was made in triplicates.

With NanoSorter®, samples containing nano-objects can be sorted from any origin, from cell culture media to biofluids. The counting module enables a rapid evaluation of the starting concentration from a reduced analysis volume (50µL). The sorting module allows rapid real time separation of fluorescent nanoparticles. After harvest, sorted nano-objects can be used for downstream analysis (imaging, DNA, RNA, proteins, lipids) or re-employed in targeted functional assays (in vitro, in vivo).

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Results

All the samples tested presented a nice and clean signal as illustrated on the recording traces (Figure 1) allowing a clear-cut detection of fluorescent events passing through the analysis canal.

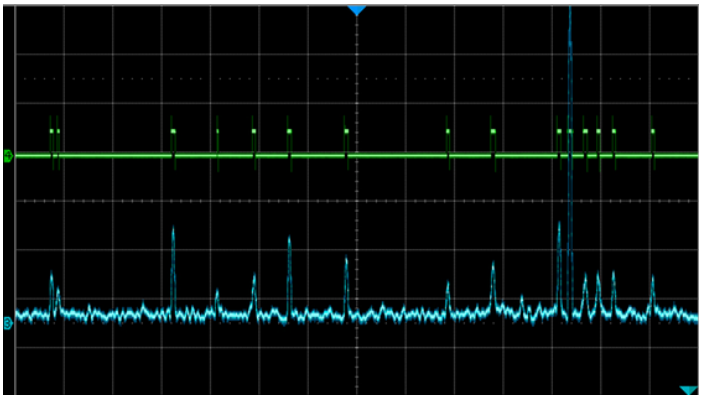
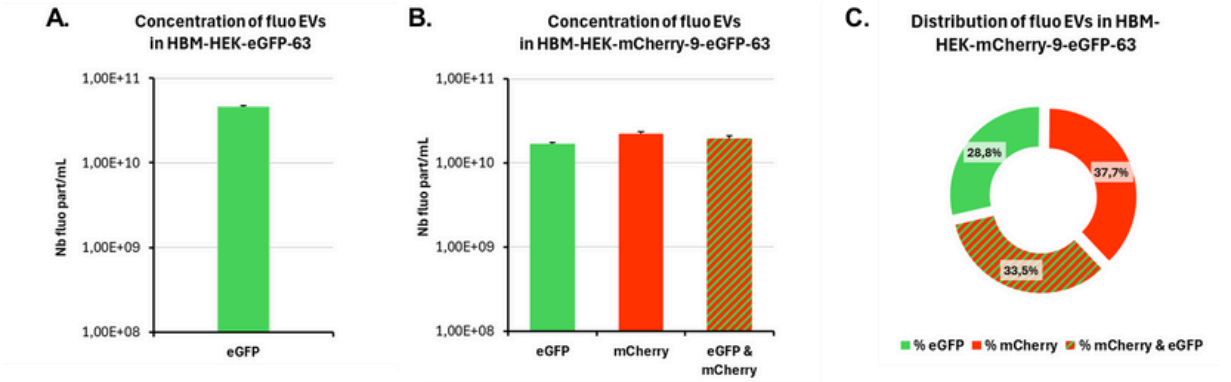


Figure 2 : (A) Oscillogram representing analog eGFP fluorescence signal (blue) and fluorescence logical detection signal (green). Timescale: 500 µs/div.

The concentrations of HEK-eGFP-63 sample and HEK-mCherry-9-eGFP-63 sample are assessed separately with single or double-laser configurations. The results, which can be seen in Figure 3, demonstrate that the concentrations comply with product specifications (>10E+9 fluo. part./ml).



	eGFP⁺
Measure 1	4,70E+10
Measure 2	4,37E+10
Measure 3	4,61E+10
mean	**4,56E+10**
SD	1,71E+09

	eGFP⁺	mCherry⁺	mCherry⁺ & eGFP⁺
Measure 1	4,70E+10	2,14E+10	2,11E+10
Measure 2	4,37E+10	2,13E+10	1,93E+10
Measure 3	4,61E+10	2,36E+10	1,86E+10
mean	**4,56E+10**	**2,21E+10**	**1,97E+10**
SD	1,71E+09	1,30E+09	1,29E+09

eGFP⁺ + mCherry⁺ + eGFP⁺ & mCherry⁺		
	mean	SD
Total concentration fluo part./mL	**5,88E+10**	1,14E+09
% eGFP	**28,8%**	1,7%
% mCherry	**37,7%**	0,7%
% mCherry & eGFP	**33,5%**	2,3%

Figure 3: Histograms representing concentration of FLUO-EVs

Conclusion

Using HEKAT Nanosorter® counting module, it has been observed that both HansaBioMed products tested are clean and present bright fluorescent signals, allowing straightforward measurement with an excellent signal/noise ratio. In these conditions, the identification of EVs fluorescent signal is quick and accurate. In accordance with HansaBioMed product specifications, both products tested showed high concentrations of fluorescent particles (>10E+9 fluo part./mL range).

HEKAT NanoSorter® core technology is sorting at high rates, allowing downstream analysis on sorted materials. In this case, a logical next step could be to selectively sort these 3 subpopulations and make tetraspanin targeted proteomic analysis to confirm proteins profile.

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