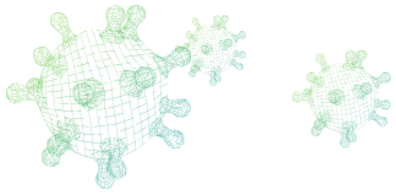
hekat fluidics

**DATASHEET**



# NANOSORTER® – SPECIFICATIONS

A single instrument to detect, count & isolate individual nanoparticles by functional criteria

- High rate (35.000 sorting decisions/s)
- High sensitivity (individual fluorescent particle counting & sorting) & reliability
- Adapted to EVs, viruses (lentiviruses, AAVs) or any fluorescent nanoparticle

NanoSorter® is a breakthrough in the field of nano-objects sorting and more generally in the field of flow cytometry. It is the first system that can detect and sort at a high-speed **individual** fluorescent nanoparticle by functional criteria (e.g. surface biomarkers).

Starting from a sample containing various nanoparticles, NanoSorter® encapsulates individual particles into **femtoliter-sized droplets** (volume 100.000 times smaller than in the existing sorting flow cytometers).

Our patented microfluidic chip generates up to **50.000 droplets per second,** and the system takes **sorting decision for each individual droplet**.

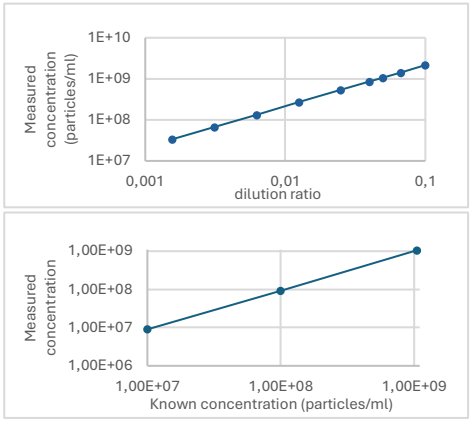
Thanks to this high decision rate, and to an ultra-sensitive optical detection, a concentrated and highly purified fraction of interest can be isolated. This sub-population can be sorted and harvested within a few hours allowing downstream analysis (genomic, transcriptomic, proteomic, lipidomic) and functional assays.

| | Specification | Counting | Sorting |
|---|---|---|---|
| **Optics** | Supported exitation wavelength | 488nm - 532nm - 561nm- 633nm - 660nm (2 lasers/device) | |
| | # of simultaneous wavelengths | 2 | |
| | Optical alignment | Automatic | |
| | Fluorescent detection | Single-Photon Counting Modules + Hardware-based particle detection algorithm | |
| | Fluorescent noise | ≤1 Hz* (False Positive) | |
| | Optical filters | User exchangeable fluorophore specific optical bandpass filters | |
| **Sample** | Particle size | < 2000nm | |
| | Maximum sample concentration | $3x10^9$ particles per ml | |
| | Minimum sample concentration | $5x10^5$ particles per ml | |
| | Volume | >50µL | |
| **Measurement** | Sheath fluid | none | Fluorinated oil with surfactant |
| | Sample flow rate | up to 30 µL/h | 3,5 µL/h |
| | Sample acquisition rate | up to 500.000 events /min | up to 2.100.000 events/min |
| | Overal concentration error | <20% | <20% |
| | Sorting error** | *na* | 0,10% |
| | Sorted particle concentration | *na* | up to $2.10^{10}$ fluo particles/ml |
| | Run time | 2 min standard (up to 15 min extent) | 1-10 hrs |
| **Data Management** | Parameters | Objects count, concentrations, subpopulations distribution | Objects count, concentrations, subpopulations distribution |
| | Data report and output files | Open file format (.pdf, .csv, .fcs) | Open file format (.pdf, .csv, .fcs) |
| | Operating system | Windows 11 Pro or newer | Windows 11 Pro or newer |
| **Physical parameters** | Dimensions | h:470 w: 600 d: 430 mm | |
| | Weight | 25 kg | |

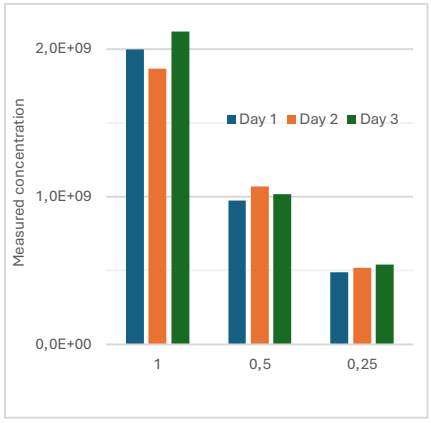
*in MiliQ @ 488nm; **estimated by sorting a 30%/70% mix of particles labeled with two diffrent fluorophores, and analysing the proportion of unwanted particles in the sorted volume

## EV Counting

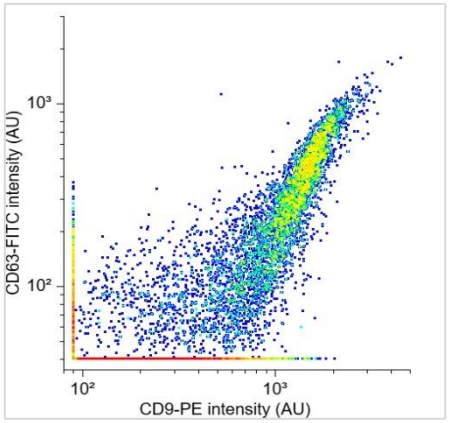
High performances, in terms of accuracy, linearity and repeatability over two wavelengths in mono and co-detection




***Linearity (top)**: tdTomato-labeled lentiviruses. Concentration measurement vs dilution.*
***Repeatability (down)**: Successive measurements over 3 days of 3 samples with relative concentrations 1, 0.5 and 0,25*



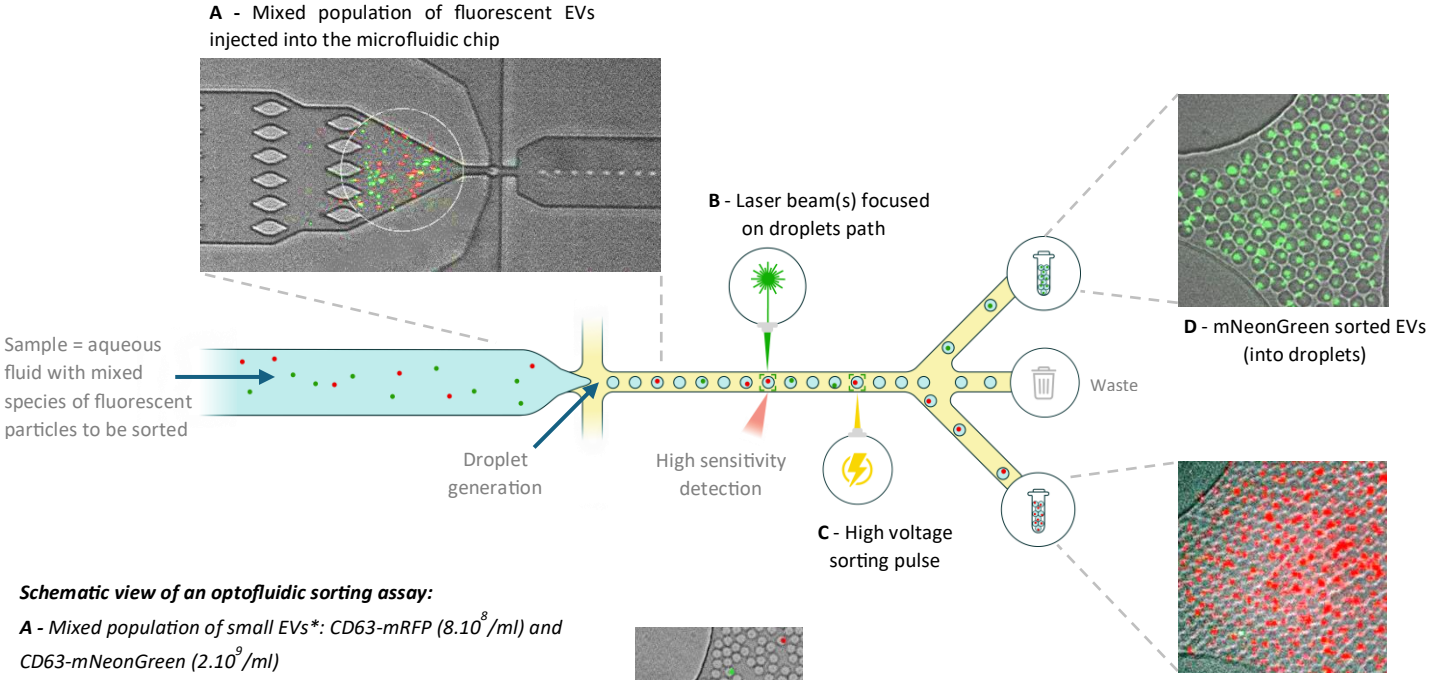

***Accuracy**: Measured concentration vs known concentration of different dilutions of 40nm beads (FluoSpheres™ orange, 2.1x10[15] particles/ml initial concentration)*




***Co-detection**: simultaneous detection and quantification of two wavelengths from serum samples stained for CD63-FITC and CD9-PE. Data obtained from a 60s run (12000 events)*

## EV Sorting

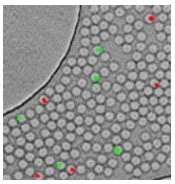
NanoSorter® can rapidly sort and concentrate subpopulations of nano-objects




***Schematic view of an optofluidic sorting assay:***

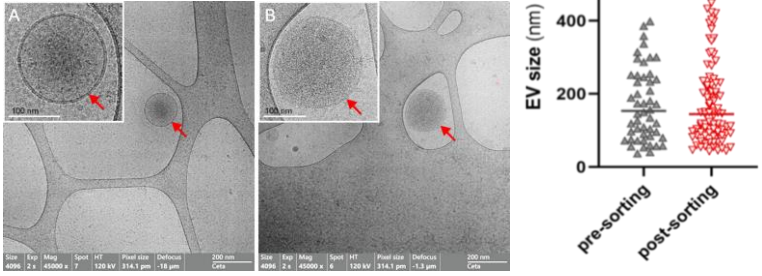
***A** - Mixed population of small EVs*: CD63-mRFP (8.10[8]/ml) and CD63-mNeonGreen (2.10[9]/ml)*
***B** - a laser beam is focused onto the droplet; fluorescence is detected with a high sensitivity detection chain.*
***C** - when a fluorescent signal of interest is detected a high voltage pulse deflects the droplets into the dedicated collection channels.*
***D** - microphotography of the "green" collected droplets.*
***E** - microphotography of the "red" collected droplets.*
***F** - collected unsorted sample.*

*After harvest, droplets are fused to obtain final subvolumes containing only the EVs populations of interest.*



**F** –Collected unsorted sample

***G** - Cryo-EM image of EVs before and after sorting demonstrate that the sorting process does not alter the EV's morphology**.*




**G** – Cryo-EM images of EVs before (A) and after (B) sorting, and particles size.

** Fluorescent EVs are provided by INSERM U1307 CRCI2NA Team EVICAT (Dr. Guillaume Van Niel) - NANTES (France).*
*** CBMN – Université Bordeaux – TALENCE (France)*