STANDARD OPERATION PROCEDURE (SOP)

#1	GENERAL INFORMATION
Procedure Title	Nanoparticle tracking analysis (NTA) protocol for scatter measurement
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#2	PROCEDURE DESCRIPTION
With the NTA in scatter mode you can determine the size distribution and particle number of nanoparticles (e.g. extracellular vesicles). General training is required before using the NTA. The training must be discussed with the person responsible for the device (Dela Khamari, dela.kh@outlook.com).	
Duration	minimum 30-45 min (depends on the number of samples)

#3	HAZARD SUMMARY
The reagents used for NTA measurement are not hazardous. The hazardousness of the samples can be judged by the user. Waste should always be treated as hazardous. Remember to use protective gloves and lab coats.	

#4	STORAGE REQUIREMENTS
The stock and 1/1000 diluted bead solution is kept at 4 °C. Allow the filtered water and PBS to warm up to room temperature, since the measurement is performed at 25 °C.	

#5	STEP-BY-STEP OPERATING PROCEDURE
1) Check if the small bottle 1 (water) and small bottle 2 (PBS) is filled and the big bottle (the waste) in the middle is empty. 2) Turn on the computer (password: 123456) and the NTA device. 3) Open the ZetaView software. 4) Wash with water by using Pump1 (5 mL/min), then inject 5 mL of 0.1 µm filtered bubble-free water to the chamber.* 5) Prepare polystyrene beads for calibration (average size: 100 nm). a) Vortex for 2 min your bead aliquote.	

b) Dilute 1 µL beads in 999 µL 0.1 µm filtered water (1/1000 dilution can be saved for about 5 days).

c) Dilute 8 µL 1/1000 diluted beads in 2000 µL 0.1 µm filtered water to get 1/250,000 diluted final concentration which can be use once.

6) Load 1-2 mL of 1/250,000 diluted beads and perform cell check, autoalignment and optimize focus.**

7) Measure the beads, with SOP PS 100 nm.

8) Wash with 0.1 µm filtered water to remove beads.

9) Wash with PBS by using Pump2 to put conductivity to 15,000 for PBS.

10) Dilute samples in 0.1 µm filtered bubble-free PBS starting by 1/1000 dilution, then do less or more based on your samples.***

11) Use SOP-PMX based on your particle size.

12) Save your folder and remove the folder from the computer on your drive each time, otherwise you risk to lose all data.

13) Finish the measurement by pumping water (Pump1) and injecting 5 mL 0.1 µm filtered water 5 times.

14) Inject air 4 times.

15) Turn off both the computer and the instrument.****

16) Empty the waste container.

17) Fill the sheet with your name and date and name of your sample in each use.

#6	NOTES

*Do not shake or vortex the samples, water and PBS to avoid air bubbles.

*To inject water and PBS, use a syringe of up to 5 mL.

**If the calibration was not successful, rinse the cell several times with water and repeat the calibration. If it is still unsuccessful, remove the solution from the cell with air and start again from step 4. If it still does not work, then a major cleaning is required, please consult with the person responsible for the device.

***Dilute samples to a minimum volume of 1 mL and inject with a 1 mL syringe. Switch on automatic temperature control so you don't have to wait for the instrument to reach the optimum temperature before each measurement (25 °C). You can use a different solution for your sample, for example physiological saline, in which case use your own buffer instead of PBS for each step.

****Please turn off the laser if the instrument is not in use for more than 15 min. Please turn off the instrument completely if the instrument is not in use for more than 2h.