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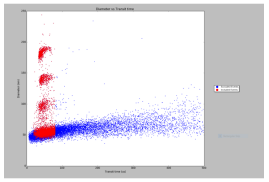
Spectradyne nCS1: Sample measurement and device maintenence protocol V.1



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Protocol status: Working
We use this protocol and it's working

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Disclaimer

This protocol summarizes key steps for a specific type of assay, which is one of a collection of assays used for EV analysis in the NCI Translational Nanobiology Section at the time of submission of this protocol. Appropriate use of this protocol requires careful, cohesive integration with other methods for EV production, isolation, and characterization.

Abstract

This is a generic protocol for using the Spectradyne nCS1 resestive pulse sensing instrument to measure the diameter distribution and concentration of nanoparticles.

Guidelines

- **The "end run" button must always be pressed before lowering the cartridge stage after a cartridge has been primed and/or a sample run on the cartridge.** If the user is uncertain about whether the "end run" button has been previously selected, it can be selected a second time. The final step of the "run cleaning cartridge" procedure involves the "end run" process so it does not need to pressed after successful completion of the cleaning procedure.
- Particles must be in solution with dilute surfactant before being analyzed on nCS1
- The "run cleaning cartridge" procedure doesn't need to be performed daily but is indicated after a long period where the instrument has not been run or any other reason where the user desires the fluidics of the instrument to be flushed.
- **The running buffer must be changed every two weeks.** It is recommended that the user empties out the old buffer, rinses with filtered/pure water and allows to dry overnight before refilling.
- Generally, used cartridges can be discarded, but **cleaning cartridges must be kept and reused**. They should be visibly marked but if they are mixed with sample cartridges can be distinguished by the intricate maze-like channels etched into the glass on their bottom side
- The full details of the preparation of sample buffer is left to the user as it has not been optimized but some potential options are to freshly filter sample buffer every other day, rinse plastics with filtered buffer before filling them, be aware of any dust or lint on surfaces used to prepare buffer, attempt to prepare buffer as sterily as possible (sterile plastics, using laminar flow hood)
- Whether or not 1% Tween in PBS affects the morphology of EVs is an area of current research and isn't yet known. To minimize surfactant effects on sample particles composed of lipids it is recommended that the time that samples are suspended in surfactant-containing buffer is minimized (prepare samples one at a time immediately before running)
- It is easier to access the sample description stored in the nCS1 cartridge logsheet than the sample description typed into the nCS1 software
- For the purposes of record-keeping, each of the 8 cartridges in one mold can be given a number to distinguish among them
- Once every month, empty water bottles and waste bottles, flush with filtered/pure water, and allow to dry overnight. Attempt to empty fluid lines attached to bottle caps
- It is possible the locking o-ring male/female fluidic connections may become damaged or worn over time and if the user is unable to connect them properly they should contact Spectradyne as having pressure leaks can affect the data
- A single cartridge may be used in a dilution experiment, working from least to most concentrated sample, aspirating sample with a pipette after it is run. Carry-over effects are undetermined and may need to be investigated. For non-dilution series, a new cartridge is needed per sample

Materials

MATERIALS

- Whatman Anotop Filter (0.02 um) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**WHA68093102
- Protein LoBind Tubes, 1.5 mL **Eppendorf Catalog #**0030108116
- Tween-20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9416
- PBS **Gibco - Thermo Fisher Scientific Catalog #** 10010023
- C-400 cartridges **Spectradyne**
- C-300 cartridges **Spectradyne**
- Protein LoBind 0.5 mL **Eppendorf Catalog #**022431064

Before start

- Ensure the USB cable is firmly plugged into both the device and PC before turning on the instrument as any slight disconnection will interput a sample run
- Check that the current software version is: 2.5.0.254
- **Check the date listed on most recent calibration file: 5/13/2020**

Preparation of running buffer

1 If running buffer is more than two weeks old, it must be prepared fresh.

2 Discard the old running buffer contained in the nCS1 buffer bottles.

3 Rinse out the buffer bottles with a small amount of purified/filtered water.

4 If there is no previously prepared 1% Tween-20 solution, prepare a 500 mL bottle of 1% Tween-20 by pipetting 5 mL of pure Tween-20 into a new sterile 500 mL bottle of DPBS.

5 Gently invert the bottle.

6 Let the bottle sit for at least 15 minutes while occasionally inverting until Tween-20 is entirely dissolved.

7 Filter half of the [M] 1 % (v/v) Tween-20 solution with a 0.2 µm PES bottle-top filter.

8 Equally divide the filtered [M] 1 % (v/v) Tween-20 solution into the two nCS1 buffer bottles.

9 Store running buffer at 4 °C and mark the date it was prepared so it can be changed in two weeks.

Device start-up

10 Turn on and log in to attached PC.

11 Obtain nCS1 buffer bottles and water bottles from the 4 °C fridge.

12 Attach the nCS1 buffer bottles to their indicated fluid lines on the side of the instrument.

Note

For all bottles, when refilling, be sure to screw the caps on tightly. Loose caps on the buffer bottles may cause pressure leaks that will result in the system from pressurizing and/or preventing chips from priming.

13 Turn on the nCS1 with the power switch on the back of the instrument.

14 Open the nCS1 software.

15 The bar at the top of the software should be green with the word "Ready" displayed. If not, shut off the nCS1, restart the PC and then repeat the power up process, ensuring the software is only opened after the nCS1 is powered on and properly connected via its USB cable.

16 If the user determines the device needs to be cleaned, obtain a cleaning cartridge.

Note

We recommend to run the cleaning cartridge at least once when turning on the instrument.

17 Properly position cartridge in slot on stage. The blue indicator light and the blue ring light should both illuminate.

18 Press the outside button to raise the stage before clicking 'run cleaning cartridge' in the software.

19 Wait about three minutes for routine to finish.

20 Lower stage by pressing metal button and return cartridge to safe storage place.

Sample preparation and loading

21 Prepare a [M] 1.0 % (v/v) solution of Tween-20 in Thermo Fisher Gibco Sterile DPBS (identical to the running buffer) or a [M] 0.1 % (v/v) solution of BSA in Thermo Fisher Gibco Sterile DPBS, ensuring ample time given to allow solids to dissolve. PBST (PBS with 1% Tween-20, filtered) should be used unless investigating buffer effects on lipid particles, as it is the default recommended buffer. Researchers with Edwin van der Pol's group have investigated other methods of removing small particles from Spectradyne sample buffers which could be considered.

22 Filter the solution with a Whatman Anotop 10, 0.02 µm syringe-driven filter into clean plastic tubes.

23 Select a cartridge type to load your sample into:

Cartridge Type	Considerations	LOD	Recommended sample concentration
C-300	Compared to C-400 there may be more cartridge-to-cartridge and mold-to-mold variability. Particles larger than ~250 nm may either clog the cartridge or simply not show up in data. Flow rate is ~10 times slower than C-400	50 nm to 55 nm when running optimally	1E10 p/mL
C-400	This is the oldest cartridge version created and has been better optimized than the C-300 meaning less cartridge-to-cartridge and mold-to-mold variability. It generally can run particles up to 400 nm, and may be able to block out rare large particles and continue running. The faster flow rate compared to the C-300 means many more particles can be measured during the same time period and recent pressure modificiations mean CVs are not significantly larger than those of the C-300.	65 to 70 nm when running optimally	5E9 to 1E10 p/mL
C-900	Untested in lab	Untested in lab	Untested in lab
C-2000	Untested in lab	Untested in lab	Untested in lab

*Note: all information in this cartridge selection table was based on experience in our lab up to May 2020, and needs to be continually re-evaluated

24 Calculate the volumes of filtered [M] 1 % (v/v) Tween/PBS, spike-in beads, and sample particles to mix together. It is suggested that the final concentation of spike-in beads be **5E9 particles/mL*,** the final concentration of sample particles be around **1E10 particles/mL*** and that the overall volume is at least 50 µL to allow sufficient mixing.

For C-300 cartridges use a 203 nm NIST PS spike-in bead

For C-400 cartridges use a 269 nm NIST PS spike-in bead

*These values were listed in May 2020 and may need to be updated at a future date based on future experience with a variety of samples

25 Thoroughly vortex spike-in beads (for example, 10 short pulses at max power) and pipette into [M] 1 % (v/v) Tween/PBS buffer. Vortex the tube thoroughly to avoid any bead aggregates.

26 Vortex sample particle sample then pipette into the buffer/bead mixture. Vortex well.

27 Obtain a new sample cartridge and a P10 pipette set to 4 µL .

28 Vortex sample particle mixture immediatlely before drawing up 4 µL of sample and inspecting filled pipette tip to ensure the absence of air bubbles.

29 Using one hand to hold the cartridge in place, insert pipette tip into the fluid loading aperature until it gently contacts the bottom of the loading chamber.

30 While slowly pulling the pipette tip upward so that it is not contacting any surface load ~ 3.5 µL of the sample into the bottom of a sample cartridge, so that a small volume of sample is left in the tip and an air bubble isn't pipetted into the cartridge fluid well.

31 Tap the cartridge against the table surface to dislodge any bubbles then hold it at eye level to inspect the fluid level and check for an even meniscus absent of any bubbles. and then insert onto cartridge stage.

32 If there is a bubble, try alternative ways of tapping the cartridge, or, use the P10 pipette to aspirate the fluid out, several times, and retry.

33 Load the cartridge onto the stage and ensure both the blue indicator dot and the blue ring light are illuminated.

Running sample on nCS1 device

34 Use the nCS1 Cartridge Log document on SharePoint to record detailed information about each sample run on the instrument. This information is critical for identifying measurement quality over time.

Note

This step is specific for the NCI Translational Nanobiology Section's use.We do, however, recommend creating a log of your cartridge and its lot number to track performance over time.

35 Enter mold ID and box ID information into the nCS1 software.

36 Enter a descriptive prefix for the sample and a sample description if desired.

37 Create a new folder on the PC to store mesaurement data with date and descriptive name. Select the new folder as the file location for the measurement.

38 Visually confirm the stage is raised so that the cartridge is properly positioned to receive fluidic inputs.

39 Press "GO!" to begin automated procedure to prime cartridge. If the instrument has not had a cleaning cartridge run, the first priming procedure will take longer than the subsequent primings. (update as of 7/27/20: if the instrument is failing to prime, press "Stop" and then press "Clear constriction" and then try priming again).

40 After priming, the instrument will automatically begin taking 10 second acquisition measurements (also known as to as "traces".)

41 The blue line indicates the instrument voltage. Sharp spikes indicated particles pasing through the nanoconstriction and red Xs indicate events that the software determines to be particle clogs of the nanoconstriction. A clog-free trace will automatically be processed by the auto-analysis engine from a raw file into a stats file, which will then be plotted as a "Live CSD." If the number of transient clogs excedes a threshold, the software will not "flag" the raw file from the trace for processing and a stats files will not be automatically generated for that trace.

42 Using the auto-analysis engine's live CSD tool, particles within a diameter range can be selected and the concentration within the range displayed. This tool can be used to

determine how long to acquire data, for example, until 10,000 spike-in beads have been measured.

Note

If the live CSD tool does not appear (may happen if the program needs to be restarted due to a crash), just click "Restart Auto-analysis Engine" under the Manual Utilities and be sure to continue processing accumulated files.

43 After the user is satisfied with the data collected, press the Stop button to end data collection.

Note

If the instrument clogs, as indicated by the recent acquisitions covered in red Xs and data not saved, click "Stop", "Clear Constriction", and then "Continuous" when allowed. This should fix most clogging situations and combine the data from the previous half of the run.

44 Press the End Run button and allow end run sequence to procede. After the sequence is finished the blue ring light should be iluminated.

45 Press the metal button, remove and discard the used cartridge.

46 Samples can continue to be run on new cartridges as described until done with nCS1 session.

Note

While the next sample is running, it possible to go to the Data Viewer software and combine the files from the previous sample acquisition to save time. To do so, select the correct folder, select all raw files from the past run, bring them down into the lower "currently loaded" box. Click "Process All" under the Process Raw Data tab. This will create stat files from the raw data. For the Translational Nanobiology Section pipeline ensure all filters are removed before clicking 'Combine Data for All Loaded' under the "Data Output" tab.

Device shut down

47 Load a cleaning cartridge onto the stage and raise the stage.

48 Select the "Instrument shut down" button and follow the on-screen instructions.

49 Remove all fluid bottles, except the waste, and return them to 4 ˚C.

Troubleshooting

50

	Problem	Solution
	After stage is lowered fluid is actively dripping onto the cartridge or stage	Immediately position a Kimwipe in the cartridge loading area to absorb fluid and turn off power switch on nCS1 which should cause fluidics lines to be clamped shut. Continue monitoring fluid dripping being caref

		ul to minimize fluid entering compartments of the device. Do not turn device back on; unplug fluid bottles, use compressed air gently on the electronics of the stage. Allow to dry overnight until turning back on to prevent any electrical shorts.
	The blue ring light doesn't illuminate after the completion of an "End Run" proecedure	Press CTRL + R to open an additional window of control buttons in

			the nCS1 softw are. Make sure that the End Run proce dure has actual ly been comp leted, if uncer tain it can be run twice. Then, chec k the chec kbox next to overi de fluid safet y contr ol and ackn olwed ge the sofw are's warni ng. Then, press the "mov e stage down to limit" butto n to lower the stage and remo	

		ve the cartridge. The fluid safety control box can remain checked as long as the user never presses the "move stage down to limit" botton unless they are certain the "End Run" procedure has been completed.
	The blue ring light and/or the blue indicator light doesn't illuminate when a cartridge is positioned on the stage	The cartridge can be moved gently back and forth on the stage to make

			sure it's positioned well. It should be able to easily slide back and forth if it is positioned flat on the stage. If this still doesn't work, the user is certain it is positioned properly and still would like to proceed, press CTRL + R to open an additional window of control buttons in the nCS1 software. Then, check the chec	

			kbox next to overide fluid safety control and acknolwedge the sofware's warning. Then, press the "move stage up to limit" button to raise the stage. The fluid safety control box can remain checked as long as the user never presses the "move stage down to limit" botton unless they are	

		certain the "End Run" procedure has been completed.
	There is an error message saying no electrical connection with cartridge	This may be caused by dust on the electrodes in the nCS1 that interface with the cartridge. A kimwipe can be used to gently wipe them down, or compressed air.
	There is an error message saying connection with device lost	This is almost always caused by the mini USB connector cable being loose. Make sure

		it is tightened and the instrument may need to be restarted.
	The nCS1 loses connection with the cleaning cartridge during instrument shut-down (blue indicator lights turn off)	This means that the automated shut-down procedure cannot be completed. Press "End Run" to drain fluid lines and prevent fluid leaks, then use "Stage Down" in the CTRL + R menu, with the fluid safety box checked, to remove cartridge. Remove

		fluid bottles and turn off instrument, being careful to watch for any fluid that may drip.