TRPS MEASUREMENT CHECKLIST

To get the best out of your TRPS instrument, please ensure that all of the steps below are completed.

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CONSUMABLES

Clean new consumables are crucial to avoid contamination.

Have you got all you need?

- [ ] New nanopore (If using a used nanopore, ensure that is has been cleaned as per the specified wash/shut down protocol and stored correctly).
- [ ] New and dust-free disposable 15 or 50 mL Falcon tubes, and Eppendorf tubes.
- [ ] New disposable single-use 10-20 mL syringe.
- [ ] New single-use filter units stored in dust-free bags.
- [ ] Clean bench area.
- [ ] Clean disposable gloves.
- [ ] New (unfiltered) pipette tips.
- [ ] Calibrated pipettes.
- [ ] Clean and dry glassware.
- [ ] Compressed filtered air or nitrogen gas.

QUALITY REAGENTS

High quality reagents are required for TRPS analysis including wetting solution, deionised (DI) water for nanopore washout, coating for nanopore protection, and electrolyte for the day's experiments.

Do your reagents meet the required standards?

- ☐ Stock solutions (PBS and coating solution) must be less than seven days old and stored at 4°C when not in use.
- ☐ Has the measurement electrolyte mix been freshly prepared today?
- ☐ Has the DI water been filtered through a new 0.22 µm filter? (This filter may be used for subsequent filtering for the day's reagent's preparation).
- ☐ Has the coating solution for the day's experiment been filtered through the 0.22 µm filter?
- ☐ Has the measurement electrolyte been filtered through the 0.22 µm filter?

SAMPLE PREPARATION: GENERAL SAMPLES

In general, please use the sample preparation methods below for TRPS analysis.

Are your samples prepared properly?

- ☐ Have all solutions been brought to room temperature before use?
- ☐ To enhance dispersion stability, consider the following:
  - Ensure pH is 7 or above
  - Ensure ionic strength is between 0.01 and 0.3 M
  - Ensure surfactant concentration is between 0.03 and 0.06%
  - Vortex samples before analysing, sonicate if necessary.
- ☐ Are the calibration standard dilutions fresh and vortexed?
- ☐ Have the samples been diluted appropriately with electrolyte?
- ☐ Are you using a good pipetting technique when diluting samples?
  - Use a calibrated pipette to dispense the larger volume of electrolyte first.
  - Ensure that there is no liquid left in the pipette before it is discarded.
  - Wipe the tip of the small pipette with a Kimwipe before dispensing the sample.
  - Ensure you have dispensed all of the sample, by mixing with the pipette.
  - Ensure you are using the smallest pipette for each volume, to minimise errors.

SAMPLE PREPARATION: BIOLOGICAL SAMPLES

For biological samples, we recommend the following purification methods for TRPS analysis.

Are your samples prepared properly?

- ☐ Has the qEV column been equilibrated with freshly-filtered stock PBS? Stock PBS solution must be less than seven days old and stored at 4 ºC when not in use.
- ☐ Have your samples been centrifuged before qEV purification?

  Have the samples been purified using qEV size exclusion columns, and purified sample volumes collected into new, clean, and dust-free tubes?
- ☐ For TRPS analysis, have the samples been concentrated, or diluted with measurement electrolyte?

Dilution may be required for EVs isolated from plasma/serum. Cell culture supernatant should always be concentrated before EV isolation and analysis.

For TRPS analysis, if the recording for a biological sample is not stable, we recommend filtering the undiluted qEV fractions through an "Ultrafree MC 0.22 µm GV Durapore spin filter unit" from Merck Millipore.

This procedure is specific to EVs and OMVs and does not apply to samples such as microvesicles (› 200 nm).

NANOPORE SETUP

Checklist for nanopore setup, coating, and sample measurement.

Have you done these checks?

Nanopore

- [ ] Are you using a brand-new or used but clean nanopore?

Wetting

- [ ] Has the nanopore been through the nanopore wetting protocol during setup?
- [ ] Before coating, establish a stable baseline current at 100-140 nA, and tap the fluid cell cap to verify this has been achieved. The baseline current should increase to a stable value, with RMS noise below 10 pA.

Coating

- [ ] Has the nanopore been through the coating protocol under maximum applied pressure for 10 minutes?

Equilibration

- [ ] Have the upper and lower fluid cells been washed out with measurement electrolyte and a stable baseline current re-achieved?

Calibration

- [ ] Have you calibrated the nanopore with Izon calibration particles at the same settings as the sample run?
- [ ] Ensure that the entire size range of calibration particles has been properly captured (blockade magnitude › 0.15 nA)

TECHNICAL TIPS

TRPS measurements are highly technical. Follow these recommendations to ensure ease of use.

Are you doing these steps?

- ☐ Use reverse-pipetting techniques for sample loading in the fluid cells
- ☐ Experienced users prefer to operate smaller pores at higher stretches rather than use low, unstable stretches with larger pores.
- ☐ Always maintain nanopore hydration during sample changeover. Letting the nanopore run dry during sample changeover may cause disruptions during subsequent measurements.
- ☐ Once the upper fluid cell has been removed, always load 35 µL of measurement electrolyte on the top of the nanopore membrane to ensure continual hydration. Wipe off just before replacing upper cell unit and add the next sample into the upper cell.

TRPS DATA COLLECTION REVIEW

Keep a detailed record of the recording parameters such as nanopore stretch, applied voltage, applied pressure, and observed baseline current while collecting data.

Are you keeping notes and reviewing collected data?

- ☐ During data recording, is the baseline current stable?
- ☐ During data recording, is the RMS noise stable and under 10 pA?
- ☐ During data recording, was a linear particle rate plot achieved? A linear rate plot indicates stable TRPS running conditions.
- ☐ During data recording, is the observed particle rate between 200-1500 particles/minute, and total particle count at least 500?
- ☐ Between each sample and calibration recording, is the baseline current variation ≤ 5% (ideal)?
- ☐ Was the average blockade magnitude for both the sample and calibration at least 0.15 nA?

NANOPORE AND INSTRUMENT CLEANING AND CARE

Follow the recommended steps below for proper cleaning and care of nanopores and the instrument.

Have you completed these steps before leaving for the day?

- ☐ Stretch the nanopore to 47 mm and replace fluid in the lower and upper fluid cells with filtered electrolyte. Apply full positive pressure and make sure no significant particles show. Replace lower and upper fluid cells with filtered DI water 2-5 times. Apply maximum pressure at the last fluid cell exchange and observe the baseline current return to 0 nA with voltage applied indicating that the nanopore is salt-free. Remove nanopore, do a final wash and dry with compressed gas before storing in a clean and dry container/bag.
- ☐ Have the fluid cells been rinsed with DI water and dried with compressed gas?
- ☐ Has the VPM nozzle been cleaned recently?
- ☐ Have the instrument and parts been decontaminated with 70% ethanol or Virkon (if applicable)?

DO NOT SOAK ANY PART OF THE qNANO SYSTEM.