

# REAGENT KIT FOR TRPS ANALYSIS

SPECIFICATIONS AND  
OPERATION GUIDE



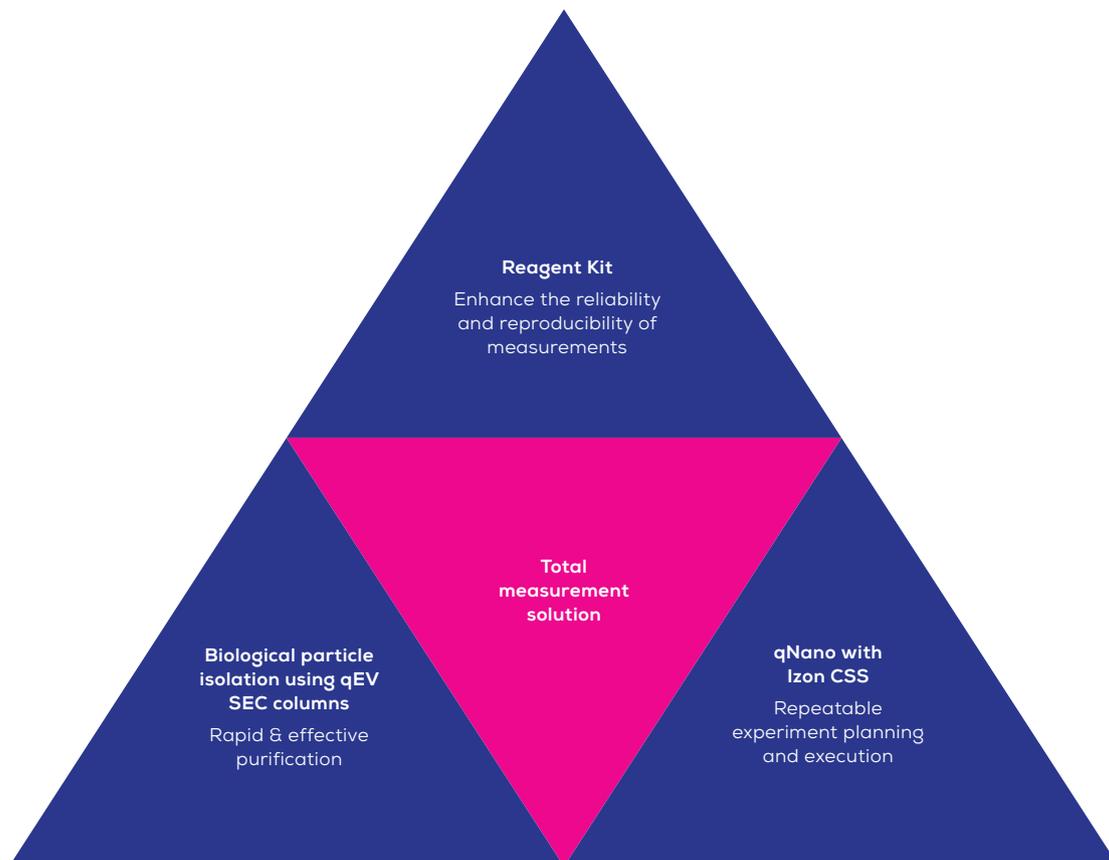
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# SPECIFICATIONS AND OPERATION GUIDE

## TOTAL MEASUREMENT SOLUTION

Izon Science's Reagent Kit is designed as part of a comprehensive measurement solution package for nano-biologicals, which includes qEV size exclusion chromatography (SEC) columns and TRPS.

- ✓ qEV SEC columns isolate extracellular vesicles (EVs) from bio-fluids, removing 99% of free protein from EV samples.
- ✓ Reagent Kits provide the components and instructions for the preparation of nanopore coating solution and contaminant-free reagents with matched pH and conductivity to ensure reliable and repeatable measurements using Izon's qNano.
- ✓ The Izon Control Suite Software (CSS) provides assistants that aid in experimental planning, capturing the required particle ranges, and performing measurements with the attention to detail necessary at the nanoscale.



**Figure 1: The Izon Total Measurement Solution ensures rapid and reliable measurements: The components of the system combine to produce high quality results.**

## APPLICABLE FOR TRPS ANALYSIS OF ALL SAMPLES AND HIGHLY BENEFICIAL FOR BIOLOGICALS<sup>1</sup>

For example, EVs derived from the following:

- ✓ Blood-derived samples (serum, plasma)
- ✓ Saliva
- ✓ Urine
- ✓ Cell culture media
- ✓ Cerebrospinal fluid

## ADVANTAGES OF USING IZON SCIENCE'S REAGENT KITS

- ✓ Reliable and consistent results; measurements on different nanopores are comparable and accurate
- ✓ Stable operation, reduces non-specific binding of sample debris and biomolecules (such as free protein) to nanopores
- ✓ Contaminant-free solutions
- ✓ Higher throughput

## SAFETY DATA SHEETS (SDS)

Safety Data Sheets for Reagent Kit components can be viewed and downloaded: <https://izon.com/sds/>

## SAFETY PRECAUTIONS

Always use appropriate personal protection such as gloves, lab coats, and safety glasses when handling reagents or qEV columns.

- ⚠ There is a trace amount of sodium azide in the wetting solution only. 0.05% w/v sodium azide is used as an anti-bacterial agent. Sodium azide in higher concentrations is toxic, direct contact with skin or eyes should be avoided.
- ✓ Waste reagents should be disposed of in a safe manner.
- ✓ Biological samples can be hazardous; consult your laboratory safety officer for information on safe handling of your sample when using a qEV column or qNano.

<sup>1</sup> / Contact Izon Support for assistance with protocols.

## STORAGE

Store the Wetting Solution Concentrate and prepared solutions at 4-8 °C. All other Reagent Kit components are stored at room temperature. Avoid leaving prepared reagent bottles open - always replace lids after use and return to the fridge as soon as possible. Observe carefully the recommended use time of prepared reagents (1 week max) and the expiry date of the wetting solution.

## OPERATING INSTRUCTIONS IN IZON CSS

Standard instructions and measurement protocols for using Reagent Kits are contained within the CSS measurement assistants. Contact [support@izon.com](mailto:support@izon.com) if you need to upgrade or require further information.

## BEST MEASUREMENT PRACTICES

Careful preparation of reagents and samples for the qNano is critical. Key considerations to get accurate and reproducible results are as follows:

- ✓ Use contaminant-free tubes and clean glassware
- ✓ Wear gloves to avoid contaminating Reagent Kit components
- ✓ Dilution errors are a common form of inaccuracy; use calibrated pipettes for dilution
- ✓ Use a new unfiltered pipette tip each time a reagent is used. This avoids contamination and ensures the longest life of the reagents
- ✓ Filter reagents daily using the 0.22 µm filters provided, to remove unwanted large contaminants
- ✓ Mix samples gently to avoid introduction of bubbles that can interfere with qNano measurements
- ✓ Use **Izon qEV SEC columns** to isolate particles of interest from biofluids, for example exosomes, microvesicles, viruses etc., to ensure quality TRPS data.

## REAGENT KIT COMPONENTS

Reagents are tailored to each stage of the measurement, to allow users to rapidly set up a stable system and ensure the measurements are run in physiologically relevant conditions. The kit contains sufficient components for four batches of reagents. The reagents included in the kit are as follows:

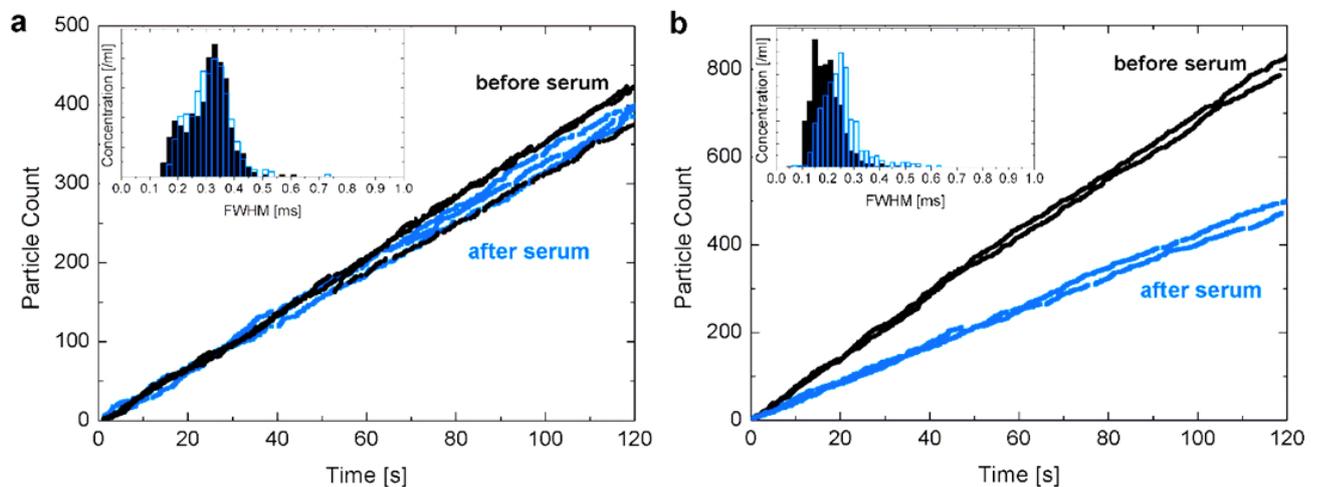
### Coating Solution (8g powder to be prepared into 4x 20mL)

A critical advantage of the Reagent Kit is the protective coating agent that minimises non-specific binding (NSB) of biological molecules/particles to the nanopore that would otherwise interfere with measurements of biological samples (Fig 2). In TRPS, NSB can interfere with measurements in two ways. Firstly, contaminating molecules can bind to the

pore and alter the membrane properties mid-measurement (reducing stability, throughput, and accuracy), and secondly, biological particles themselves can bind to the membrane, causing it to block and preventing further measurements.

Blockade frequencies and FWHM durations of calibration particles remained constant before and after serum runs, when pore coating was applied (Fig 2a). Without coating, a decrease in blockade frequency and increase in FWHM (Fig 2b) suggests pore modification through macromolecules such as proteins from serum. When pores are modified mid-measurement by NSB debris, it is difficult to achieve consistent results.

qEV purification greatly reduces the problems caused by NSB; however, pre-treatment of the nanopore is necessary to prevent NSB of low-level contaminants that may remain after purification.



**Figure 2:** Calibration particles were recorded before running a protein-rich sample (black) and after the sample (red), in order to assess the effect of pore modification processes on the stability of a pore during measurement (data was taken from Vogel, R., et al., 2016). Blockade rates and mean full width half maximum (FWHM) durations (inset) of 200 nm calibration particles with (a) and without (b) coating of the pore.

- ✔ Store the powder well sealed at room temperature.
- ✔ Shelf life: 1 week when made up as a solution, if stored at 4-8°C



Glassware for mixing PBS must be clean and devoid of particulates, washed with deionised (DI) water. Use high quality plasticware for reagent mixing and sample preparation e.g. 1.5 mL, 15 mL, 50 mL plastic tubes, we recommend the use of Axygen Scientific tubes.

#### PBS Tablets (4 tablets)

The supplied PBS tablets are used to prepare stock PBS solutions solely for TRPS and qEV columns. Reagent quality is highly important for TRPS. **Do not use general purpose lab PBS, as it may contain particulates and microbial contaminants that block nanopores.** Stock PBS is used as the measurement electrolyte (ME), which is also used as the diluent for samples and calibration particles. If using a buffer other than PBS, substitute the alternate buffer where ME is mentioned in the steps below.

- ✓ Store at room temperature.
- ✓ Shelf life: 1 week as a solution, if stored at 4-8°C

#### Wetting Solution Concentrate (4 mL)

Wetting solution concentrate is a surfactant solution, to be added to the PBS stock solution to make ME. Adding a surfactant to particle suspensions via the measurement electrolyte helps wet the pore, keep it clean, maintain system stability, and reduce the rate of particle aggregation.

- ✓ Store at 4-8°C
- ✓ Shelf life: 6 months once opened, if stored at 4-8°C

#### Syringe Filter Unit, 0.22 µm (20 pieces) and 0.45 µm (4 pieces)

Single use 0.45 µm filters are provided to filter the coating solution when it is first prepared (Step 2 below). Single use 0.22 µm syringe filters are provided to filter DI water for flushing, measurement electrolyte and wetting solution sequentially before use each day. A critical part of the measurement of nanoparticles is high-purity reagents. It is necessary to filter all solutions to remove any large particles that will block the nanopore.

- ✓ Store well sealed at room temperature.

## Solution Preparation

All unused stock solutions should be stored in the fridge and discarded after one week.

<b>1 / Prepare Measurement Electrolyte</b>	<p>Make up a fresh batch of <b>Measurement Electrolyte (ME)</b> <b>weekly</b> and filter 15 mL daily with a 0.22 µm syringe filter before use.</p> <ul style="list-style-type: none"><li>- Rinse and clean glass bottle with deionised (DI) water. *</li><li>- Completely dissolve one PBS tablet in 200 mL of DI water.</li><li>- Add 600 µl of Wetting Solution Concentrate to the PBS solution and swirl gently to mix.</li><li>- Seal container and label, along with the date.</li><li>- Always allow solutions to warm up to room temperature before use.</li></ul>
<b>2 / Prepare Wetting Solution</b>	<p>Make up a fresh batch of Wetting Solution <b>weekly</b> and filter <b>daily</b> with a 0.22 µm syringe filter before use.</p> <ul style="list-style-type: none"><li>- Add 9.9 mL of ME to a 15 mL falcon tube.</li><li>- Add 100 µL of Wetting Solution Concentrate.</li><li>- Seal container and label, along with the date.</li></ul>
<b>3. Prepare Coating Solution**</b>	<p>Make up a fresh batch of Coating Solution <b>weekly</b> and filter 1 mL <b>daily</b> with a 0.22 µm syringe filter before use.</p> <ul style="list-style-type: none"><li>- Add 15 mL of <b>ME</b> to a 50 mL tube and place in a warm water bath.</li><li>- Accurately weigh out 2.0 g of the Izon coating solution powder and slowly add to the warmed <b>ME</b>. Fit the lid tightly and mix vigorously until the lump of powder has disappeared. Leave to sit in the water bath until the solution is clear.</li><li>- When the solution is clear top up with ME to make a total of 20 mL.</li><li>- Filter with a 0.45 µm filter into a clean labelled tube, along with the date.</li></ul>

\* DI water should be high quality with resistivity of approximately 18 MΩcm-1. Water should be filtered with a 0.22 µm syringe filter.

\*\* For biological samples only, such as samples containing proteins, lipids and other biomolecules.

## PARTICLE PREPARATION

### Calibration Particles

All calibration particles must be diluted from concentrated stocks immediately before use, using Measurement Electrolyte. Each nanopore size has an associated target particle concentration. Consult the Nanopore Reference Guide in the qNano User Manual for further details.

### Sample Particles

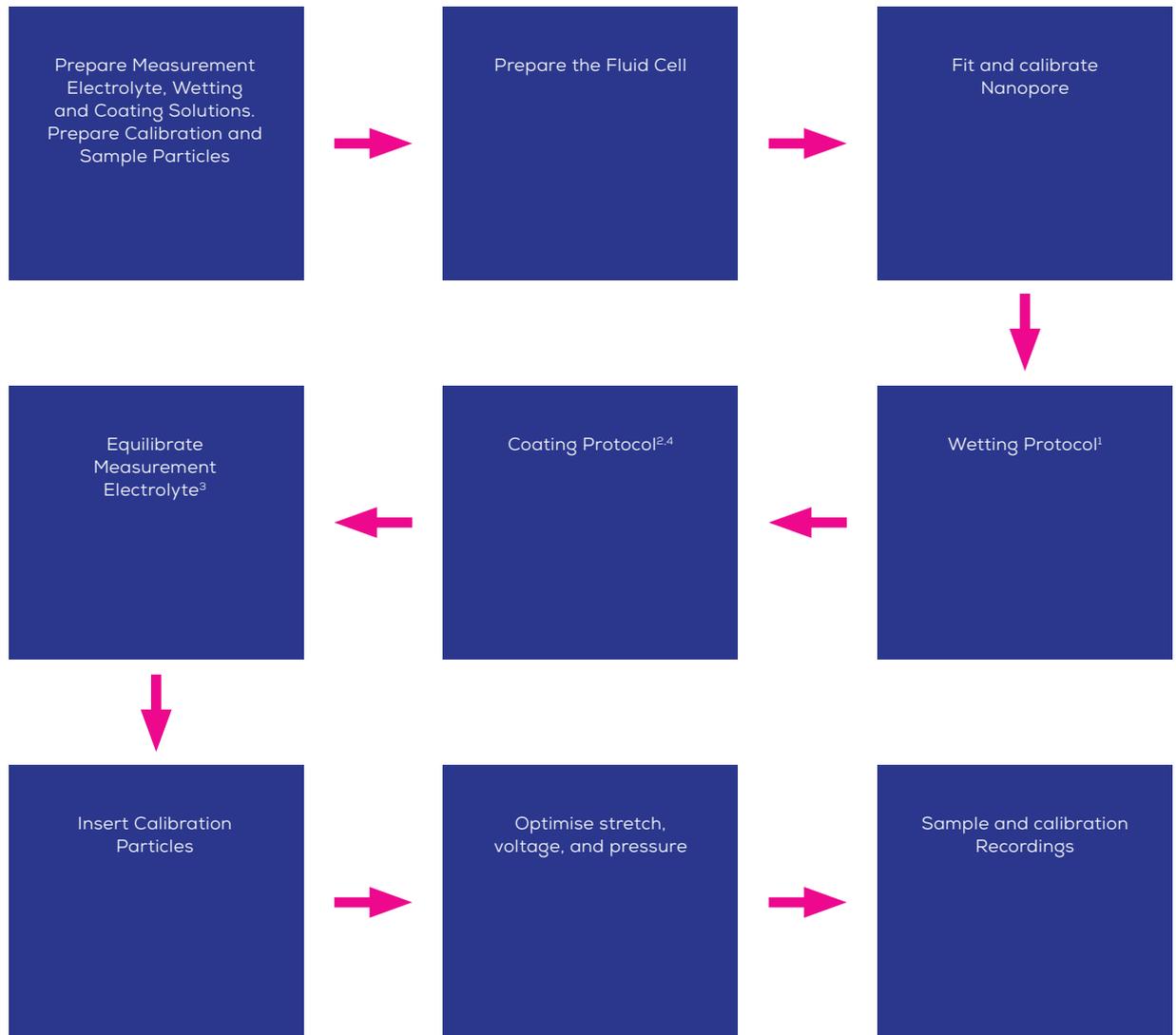
Correct sample preparation will provide faster and more accurate results. For the best results Izon Science recommends the use of qEV separation columns for biological samples. Each sample may require slightly different preparation.

- ✓ Follow the protocol outlined in the Izon qEV Technical Note to isolate the EVs from the raw sample. When using qEV separation columns, reagent quality is critical. Izon recommends the use of Sigma-Aldrich PBS tablets (Cat# P4417) with DI water.
- ✓ Dilute the qEV fraction(s) in **Measurement Electrolyte**. The dilution should be optimised to achieve a particle rate at the highest operating pressure of 200-1500/ min to avoid pore blockage and particle coincidence events.

If an initial approximate concentration of the sample is unknown, a series of samples may be prepared at different dilutions, e.g. 1:100, 1:10, 1:5. For EV samples from qEV purified plasma we recommend 1:5 to 1:10 initially.

- ✓ **Highly polydisperse samples:** In most cases EV samples have been centrifuged prior to qEV purification. However, if the sample contains very large contaminants that create problems for TRPS analysis, consider filtering the sample. Izon recommends Millipore spin filters (Ultrafree - MC centrifugal filters). Note: as these remove larger particulates the data will be biased to some degree. Contact support@izon.com for advice.

## NANOPORE SETUP OVERVIEW



Users should complete an Izon Training Programme before attempting to measure their own samples. The chart below shows an overview of Nanopore Setup:

### 1 / WETTING PROTOCOL

- ✓ Load filtered wetting solution into the lower (75 µL) and upper (35 µL) fluid cells.
- ✓ Apply maximum pressure of 20 mbar for at least 4-5 minutes or until a stable baseline current can be observed.
- ✓ Verify that the nanopore is wetted by applying an appropriate voltage and check for a stable baseline current (around 140 nA; RMS noise < 10 pA).



If there is difficulty establishing a stable baseline then an Izon PAD can be used to apply pulses of pressure, try this up to 10X before attempting to click the fluid cell cap.

- ✓ Remove the wetting solution from the bottom fluid cell and add 75 µL of ME to the lower fluid cell and 35 µL of ME to upper fluid cell. Ensure that the baseline returns to a stable condition.

### 2 / COATING PROTOCOL\*

Load filtered Coating Solution in the upper (35 µl) and lower (75 µl) fluid cells, the current should be about 2/3 of what

- ✓ was established in the previous step.

Apply maximum pressure for 10 minutes.

\* Pore coating is only required for biological samples.

### 3 / EQUILIBRATE MEASUREMENT ELECTROLYTE

- ✓ Flush the coating solution out of the upper and lower fluid cells two to three times with ME solution before adding ME to the top and bottom fluid cell.
- ✓ Apply maximum pressure of 20 mbar for approximately 1 minute, or until the baseline is no longer drifting.

### 4 / RECOATING THE PORE

- ✓ The pore coating is stable for at least 2 hours, after that the pore needs to be recoated.
- ✓ Wash out the pore with fresh Measurement Electrolyte at a pressure of 20 mbar for at least 1 min.
- ✓ Repeat the coating protocol (see #2)
- ✓ After equilibrating with Measurement Electrolyte, the pore is ready for sample measurements again.

## REFERENCES

Vogel, R., et al., A standardized method to determine the concentration of extracellular vesicles using tuneable resistive pulse sensing. *Journal of Extracellular Vesicles*, 2016. 5(1): p. 31242.



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