REAGENT KIT
FOR TRPS ANALYSIS

SPECIFICATIONS AND OPERATION GUIDE

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SPECIFICATIONS AND OPERATION GUIDE

TOTAL MEASUREMENT SOLUTION

Izon Science's TRPS 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. The kit is intended to be used in research laboratories by professional personnel for research use only.

- qEV SEC columns isolate extracellular vesicles (EVs) from biofluids, removing 99% of free protein from EV samples.
- The TRPS Reagent Kit 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 TRPS intruments.
- The Izon instruments provide integrated measurement assistance to aid in experimental planning, capturing the required particle ranges, and performing measurements with the attention to detail necessary at the nanoscale.

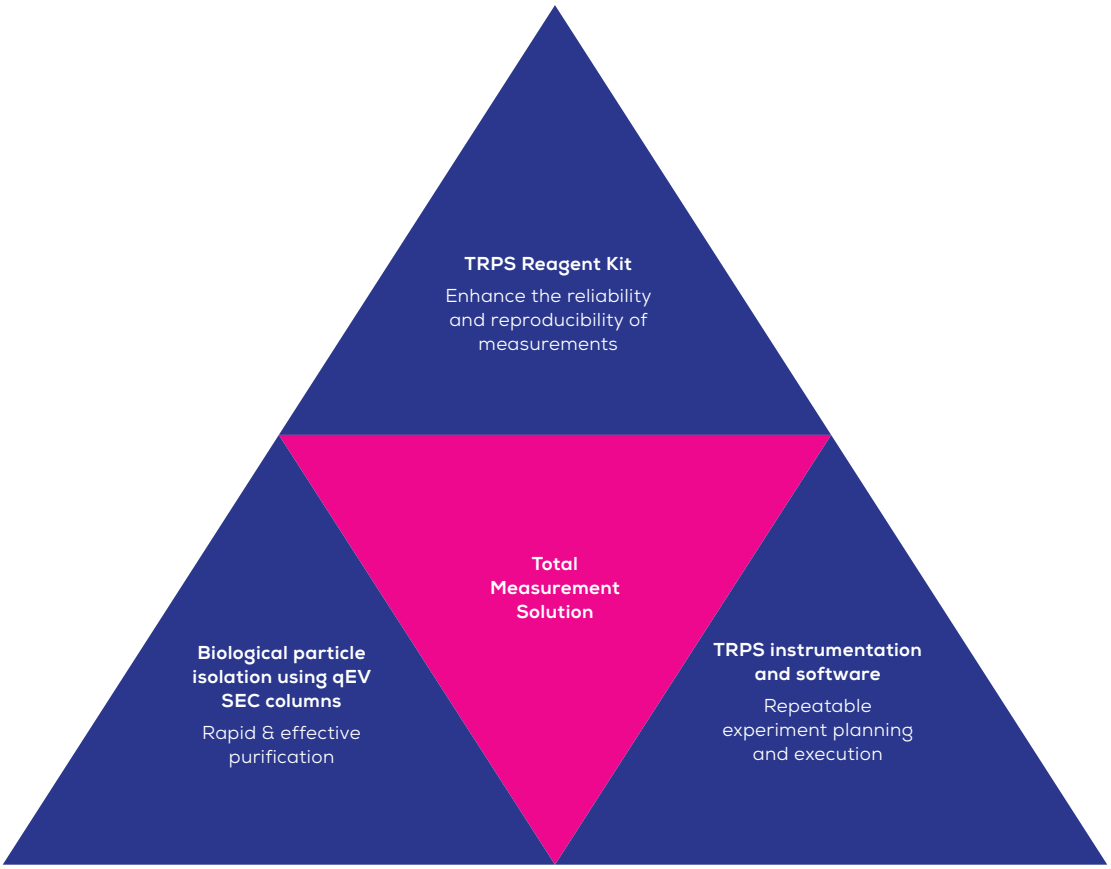

Figure 1: The Izon Total Measurement Solution ensures rapid and reliable measurements: The components of the system combine to produce high quality results.

THE TRPS REAGENT KIT IS RELEVENT FOR THE ANALYSIS OF ALL SAMPLE TYPES, AND IS PARTICULARILY BENEFICIAL WHEN WORKING WITH BIOLOGICAL SAMPLES[1]

For example, for EV-containing samples such as:

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

ADVANTAGES OF USING THE TRPS REAGENT KIT

- 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://support.izon.com/safety-data-sheets

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 TRPS instrument.

1 / 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

Reagents prepared in this kit are used for TRPS measurement protocols, and will be referred to in the software as Measurement Electrolyte (ME), Wetting Solution (WS) and Coating Solution (CS). Visit support.izon.com for the latest software and protocols.

BEST MEASUREMENT PRACTICES

Careful preparation of reagents and samples for TRPS 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 TRPS 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 (8 g powder to be prepared into 4x 20 mL)

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

Blockade frequencies and Full Width Half Maximum (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 the pore may be modified by macromolecules, such as serum proteins. 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.

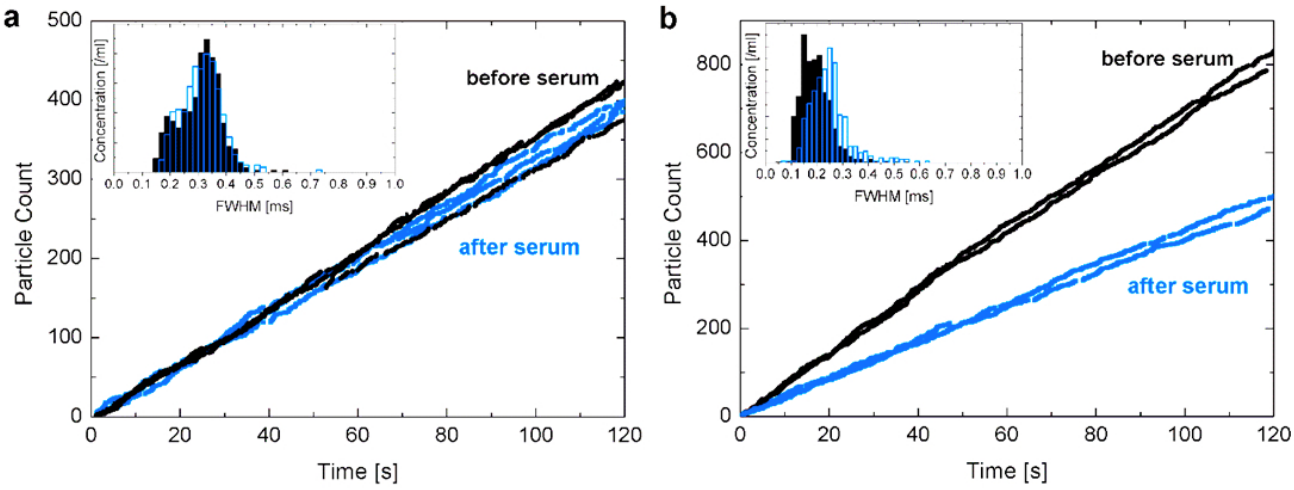

Figure 2: Calibration particles were recorded before running a protein-rich sample (black) and after the sample (blue), in order to assess the effect of pore modification processes on the stability of a pore during measurement (Vogel, R., et al., 2016). Blockade rates and mean FWHM durations (inset) 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. Izon recommends 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, wetting solution and coating solution (if required) 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.

1 / Prepare Measurement Electrolyte	Make up a fresh batch of stock **Measurement Electrolyte (ME) weekly** and filter 15 mL daily working volume with a 0.22 µm syringe filter before use. – Rinse and clean glass bottle with deionised (DI) water.* – Completely dissolve one PBS tablet in 200 mL of DI water. – Add 600 µL of Wetting Solution Concentrate to the PBS solution and swirl gently to mix. – Seal container and label, along with the date. – Always allow solutions to warm up to room temperature before use.
2 / Prepare Wetting Solution	Make up a fresh batch of stock Wetting Solution **weekly** and filter 2 mL working volume **daily** with a 0.22 µm syringe filter before use. – Add 9.9 mL of unfiltered, stock ME to a 15 mL falcon tube. – Add 100 µL of Wetting Solution Concentrate. – Seal container and label, along with the date.
3. Prepare Coating Solution**	Make up a fresh batch of stock Coating Solution **weekly** and filter 1 mL working volume **daily** with a 0.22 µm syringe filter before use. – Add 15 mL of **ME** to a 50 mL tube and place in a warm water bath. – Accurately weigh out 2.0 g of the Izon coating solution powder and slowly add to the warmed **ME.** 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. – When the solution is clear top up with ME to make a total of 20 mL. – Filter with a 0.45 µm filter into a clean tube and label, along with the date.

* DI water should be high quality with resistivity of approximately 18 $M\Omega cm^{-1}$. 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 homogenised by vortexing for 10 seconds, then 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 your TRPS system 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 user manuals 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

To set up a nanopore before proceeding to TRPS analysis, complete the following steps until a stable baseline has been re-established. In the Exoid Control Suite (ECS) software, these steps are provided on the screen as a walkthrough and contain some extra characterisation steps to make measurements easier further along the track. This guide should be followed in the event that manual nanopore setup is required, for example on the qNano.

Prepare the solutions

- Prepare Measurement Electrolyte (ME), Wetting, and Coating solutions.
- Prepare calibration and sample particles.

Prepare the Fluid Cell

- Soak lower fluid cell for 15 mins in 70% EtOH.

Load the Nanopore

- Fit the arms of the nanopore onto the stretcher jaws and apply a stretch of 47 mm. On the Exoid, calibration of the stretch is automatic. qNano requires manual calibration.

Wetting Protocol

- Load wetting solution into the fluid cell and apply maximum pressure for 2 minutes.

Coating Protocol (Biologicals Only)

- Load coating solution into the fluid cell and apply maximum pressure for 10 minutes.

Equilibrate Baseline

- Load ME into the fluid cell and establish a stable baseline.

Insert Calibration Particles

- Load 35 µL of calibration particles into the upper fluid cell.

Optimise Parameters

- Adjust voltage, pressure and stretch to achieve current of 120-140 nA, blockade size of 0.2-2 nA and particle rate of 200-1500 particles per minute.

Proceed to TRPS Analysis

- Run calibration and samples as required to complete TRPS analysis.

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 for at least 2 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 use an Izon Pressure Application Device (PAD) to apply pulses of pressure, try this up to 10x.

- 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 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 maximum pressure for at least 1 minute.
- 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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