TRPS REAGENT KIT

SPECIFICATIONS AND PREPARATIONS GUIDE

IZON

www.izon.com

Izon Science Limited
PO Box 9292
Addington
Christchurch 8024
New Zealand

Telephone: +64 3 357 4270
Email: support@izon.com
Website: www.izon.com

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1 / TRPS REAGENT KIT OVERVIEW

1.1 Overview

The TRPS Reagent Kit provides the components and instructions for the preparation of solutions used for TRPS measurements. TRPS reagents are contaminant-free with matched pH and conductivity to ensure reliable and repeatable measurements.

Advantages of using the TRPS Reagent Kit include:

- Reliable and consistent results - measurements on different nanopores are comparable and accurate.
- Stable operation - reduced non-specific binding of sample debris and biomolecules (e.g., free protein) to nanopores.
- Contaminant-free solutions if made and stored correctly.
- Compatible with all sample types, including biological samples.

1.2 Intended Use

The TRPS Reagent Kit is intended for use by professional personnel only.

1.3 TRPS Workflow

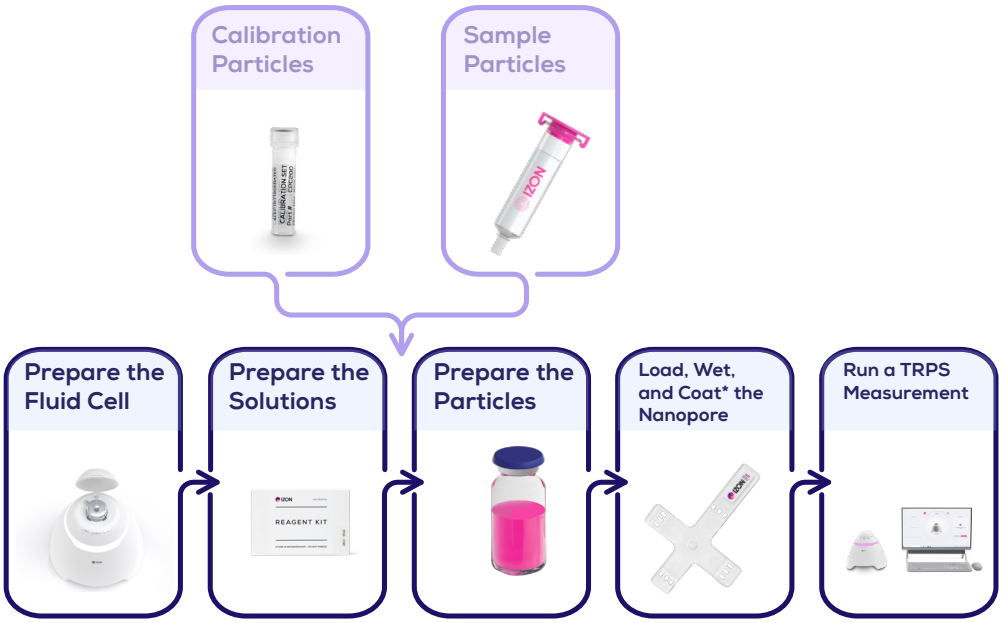

Figure 1: Overview of the TRPS workflow. *Coating is only applicable for biological samples and for particles prone to binding to plastic.

2 / SAFETY AND BEST PRACTICE

Safety Data Sheets for TRPS Reagent Kit components, qEV columns, and other relevant components can be found at support.izon.com/safety-data-sheets

2.1 Safety Precautions

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

- The Wetting Solution contains trace amounts of sodium azide (0.05% w/v), used as an anti-bacterial agent. Sodium azide is toxic in higher concentrations; avoid direct contact with skin or eyes.
- Waste reagents should be disposed of in a safe manner. All stock solutions not used within one week should be discarded according to local guidelines.
- 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.

2.2 Best Measurement Practices

Careful preparation of reagents and samples for TRPS is essential for achieving accurate and reproducible results. The following recommendations should be adhered to:

- Use clean, contaminant-free glassware.
- Wear gloves to avoid contaminating the TRPS Reagent Kit components.
- Use calibrated pipettes for dilution steps and check dilution calculations, as these are a common form of inaccuracy.
- Use a new unfiltered pipette tip each time a reagent is used to avoid contamination and optimise reagent life.
- Filter reagents daily using the 0.22 µm filters provided to remove large contaminants.
- Mix samples gently to avoid introduction of bubbles that can interfere with TRPS measurements.
- To obtain a highly purified sample for TRPS measurement, use qEV columns to isolate particles of interest.

3 / COMPONENTS AND STORAGE

The TRPS Reagent Kit includes key components for operating Izon's TRPS instruments.

Coating Solution (CS) Powder

The Coating Solution minimises non-specific binding of particles to the nanopore surface. It is needed for biological samples only, i.e. those containing proteins, lipids and other biomolecules.

- 8 g powder to be prepared into 4 x 20 mL solutions.
- Store the 8 g powder well sealed at room temperature.
- Once made into a solution, store at 4-8 °C and use within one week.

PBS Tablets

The supplied PBS tablets are used to prepare stock PBS solutions for TRPS measurement. Do not use general purpose lab PBS as it may contain particulates and microbial contaminants that block nanopores and affect your measurements.

Stock PBS is used in the Measurement Electrode (ME), which is also used as the diluent for samples and calibration particles. If using a buffer other than PBS, replace ME with the alternative buffer in the steps outlined in this document and TRPS user manuals.

- 4 tablets: store at room temperature.
- Once made into a solution, store at 4-8 °C and use within one week.
- Glassware for mixing PBS must be clean, devoid of particulates, and 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.

Wetting Solution (WS) Concentrate

Wetting Solution concentrate is a surfactant solution that is used to make ME and WS. Adding a surfactant to particle suspensions via the ME helps wet the pore, keep it clean, maintain system stability, and reduce particle aggregation.

- 4 mL solution to be stored at 4-8 °C and used within 6 months of opening.
- Once WS is made up, store at 4-8 °C and use within one week.

Syringe Filter Unit

Using pure and uncontaminated reagents is critical for accurate nanoparticle measurement and preventing nanopore blockages. To enable filtration, the following components are included:

- **Single-use 0.22 µm filter (x 20 units):** Used to filter DI water for flushing, ME, WS and CS (if needed), sequentially before use each day.
- **Single-use 0.45 µm filter (x 4 units):** Used to filter the CS when it is prepared.

Store filter units well sealed at room temperature.

Table 1: Kit Component Quantity and Storage Instructions

<table>
<tr><th>Kit Component</th><th>Quantity</th><th colspan="2">Storage Instructions</th></tr>
<tr><td>PBS tablets</td><td>4</td><td rowspan="3">Store at room temperature (15 – 25 °C)</td><td rowspan="3"> 
</td></tr>
<tr><td>Syringe filter (13 mm x 0.22 µm)</td><td>20</td></tr>
<tr><td>8.5 g Coating Solution powder</td><td>1</td></tr>
<tr><td>Syringe filter (25 mm x 0.45 µm)</td><td>4</td><td></td><td></td></tr>
<tr><td>Wetting Solution concentrate</td><td>1</td><td>Once opened, store at 4-8 °C</td><td> 
</td></tr>
<tr><td> 
</td><td colspan="3">Once the Wetting Solution, Coating Solution and Measurement Electrolyte solution have been prepared, store at 4-8 °C.</td></tr>
</table>

4 / SOLUTION AND PARTICLE PREPARATION

4.1 Solution Preparation

The following information provides instructions on preparation protocols and their recommended frequency of use.

Measurement Electrolyte

1. Rinse and clean glass bottle with DI water.
2. Completely dissolve 1 x PBS tablet in 200 mL of DI water.
3. Add 600 µL of WS concentrate to the PBS solution and swirl gently to mix.
4. Seal and label the container (include the date).
5. Always allow solutions to warm up to room temperature before use.

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.

- Weekly: Make up a fresh batch of stock ME.
- Daily: Before use, filter 15 mL working volume with a 0.22 µm syringe filter.

Wetting Solution

1. Add 9.9 mL of unfiltered stock ME to a 15 mL Falcon tube.
2. Add 100 µL of WS concentrate and swirl gently to mix.
3. Seal and label the container (include the date).

- Weekly: Make up a fresh batch of stock WS.
- Daily: Before use, filter 2 mL working volume with a 0.22 µm syringe filter.

Coating Solution (for use with biological samples or particles that are likely to bind to plastic)

1. Add 15 mL of ME to a 50 mL tube and place in a warm water bath.
2. Accurately weigh out 2.0 g of CS powder and slowly add to the warmed ME.
3. Fit the tube lid tightly and mix vigorously until the powder has dissolved.
4. Leave the tube to sit in the water bath until the solution is clear.
5. Top up the solution with ME, to a total of 20 mL CS solution.
6. Filter with a 0.45 µm filter into a clean tube and label it with the date.

- Weekly: Make up a fresh batch of stock CS.
- Daily: Before use, filter 1 mL working volume with a 0.22 µm syringe filter.

Once complete, your TRPS reagents are ready to be used according to your TRPS instrument training user manual.

4.2 Particle Preparation

Calibration Particles

Calibration particles must be homogenised by vortexing for 10 seconds, then diluted from concentrated stocks immediately before use, using ME. Each nanopore size has an associated target particle concentration. Refer to the support article below for further details:

https://support.izon.com/how-do-i-know-what-nanopore-is-suitable-for-my-sample

Sample Particles

Prepare your sample for analysis by purifying and diluting it appropriately. For biological samples, we recommend the use of qEV columns.

For the study of EVs, follow the protocol outlined in the qEV user manual. When using qEV columns, we recommend the use of Sigma-Aldrich PBS tablets (Cat# P4417) with DI water as a buffer.

- Prior to TRPS measurement, dilute your sample in ME. 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 starting with dilutions of 1:5 to 1:10.
- 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 our support team for advice.

5 / NANOPORE SETUP

Complete the Izon Training Programme before attempting to measure your own samples.

5.1 Nanopore Setup Overview

To prepare a nanopore for TRPS analysis, establish a stable baseline current by following the on-screen instructions in the Exoid Control Suite software. If manual nanopore setup is required, refer to the Nanopore Setup Guide which provides an overview of the preparation steps involved:

support.izon.com/nanopore-setup-guide

1. Nanopore Wetting

Nanopore wetting is an important step in setting up your nanopore for TRPS measurement. For a step-by-step guide, refer to the Nanopore Wetting Guide:

support.izon.com/nanopore-wetting-guide

2. Nanopore Coating

Nanopore coating is required for biological samples only. Follow the steps below:

a. Load filtered Coating Solution in the upper (35 µL) and lower (75 µL) fluid cells, the current will decrease to approximately 2/3 of what was established in the wetting step.

b. Apply maximum pressure for 10 minutes.

3. Equilibrate Baseline

a. 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.

b. Apply maximum pressure for approximately 1 minute, or until the baseline is no longer drifting.

4. Recoating the Nanopore

a. The nanopore coating is stable for at least 2 hours; after that the pore needs to be recoated.

b. Wash out the pore with fresh Measurement Electrolyte at maximum pressure for at least 1 minute.

c. Repeat the coating protocol (see #2).

d. After equilibrating with ME, the pore is ready for further sample measurements.

9 / RESOURCES

For access to the Exoid user manual and other guides, visit support.izon.com/getting-started-with-the-exoid

Additional support material is available at support.izon.com

If you have any questions that are not answered on the support portal, or your instrument requires repairs/ maintenance, please contact our support staff via the online support portal by raising a support ticket.


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