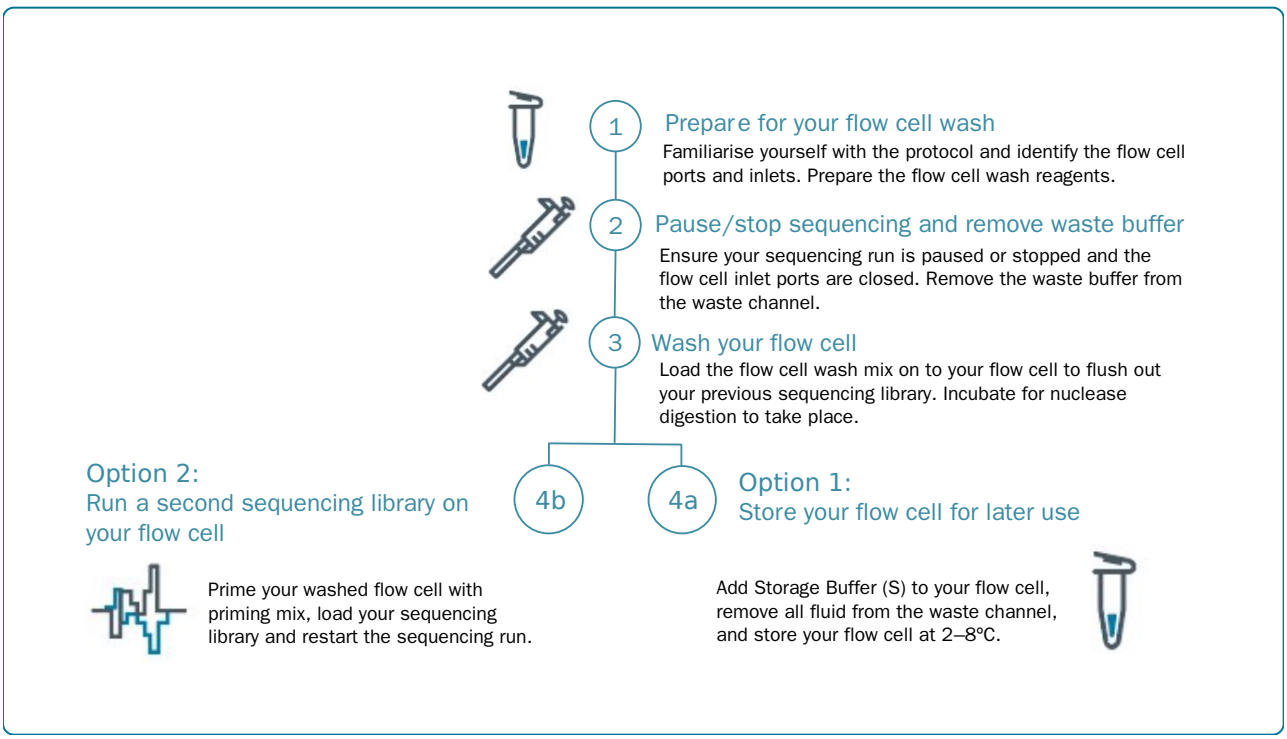
Flow Cell Wash Kit
EXP-WSH004
(PromethION flow cell)

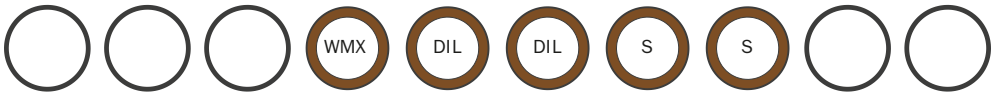


Wash = DNase I digestion of the loaded library; a flow cell can typically be washed and re-used 3–6 times.

After the wash: Option 1 (usual) – Storage Buffer, keep at 2–8°C (Step D); Option 2 – load a new library straight away (S

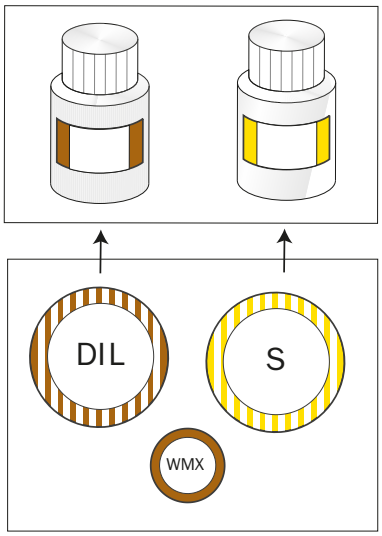
Source: Oxford Nanopore Technologies, Flow Cell Wash Kit (EXP-WSH004 or EXP-WSH004-XL), PromethION, WFC_9120_v1_revS_25Jul2025. Volumes, times and figures are unchanged; layout is the lab bench format.

EXP-WSH004 kit



WMX : Wash Mix
DIL : Wash Diluent
S : Storage Buffer

EXP-WSH004-XL kit



WMX : Wash Mix
DIL : Wash Diluent
S : Storage Buffer

WMX : Wash Mix – contains DNase I. **Keep on ice, do NOT vortex.**

DIL : Wash Diluent – exonuclease buffer that maximises DNase I activity. Thaw at RT, vortex, spin down.

S : Storage Buffer – lets a washed flow cell be stored at 2–8°C until its next use.

Compatibility of this protocol

This protocol should only be used in combination with:

1. Flow Cell Wash Kit (EXP-WSH004 or EXP-WSH004-XL)
2. PromethION flow cells, R10.4.1 and R9.4.1
3. RNA flow cells: library is flushed, pores are not restored

Materials :

1. Flow Cell Wash Kit (EXP-WSH004 / EXP-WSH004-XL)
2. Flow cell priming reagents FCF + FCT (Kit 14): from the sequencing kit, EXP-AUX003 or EXP-FLP004
3. Sequencing Auxiliary Vials V14 (EXP-AUX003): SB, EB, LIS, LIB, FCF, FCT – for re-loading a library
4. New or stored DNA library (Option 2 only)

Consumables :

1. 1.5 ml Eppendorf DNA LoBind tubes
2. Ice

Equipment :

1. PromethION device (flow cell stays in the device)
2. P1000, P200 and P20 pipettes and tips
3. Vortex mixer
4. Microfuge
5. Ice bucket with ice
6. Timer

Step A. Prepare the Flow Cell Wash Mix

※ **Prewarm :** a. Wash Mix (WMX) **on ice – do NOT vortex**
b. Wash Diluent (DIL): thaw at RT, **vortex**, spin down briefly, place on ice
c. Ice bucket with ice

※ **Prepare the wash mix fresh for each use**; do not store the prepared Flow Cell Wash Mix for > 1 day.
※ If the loaded library is to be recovered from the flow cell, **do that first** (ONT library recovery protocol).

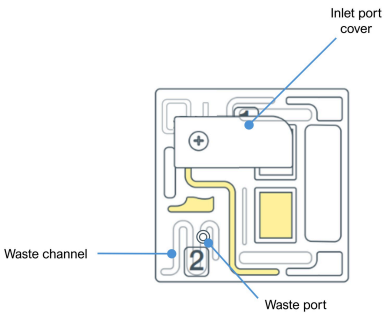
1. In a fresh 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:

Reagent	**Volume per flow cell**
Wash Mix (WMX)	**2 µl**
Wash Diluent (DIL)	**398 µl**
Total volume	**400 µl**

2. Mix well by pipetting. **Do NOT vortex** the Flow Cell Wash Mix. Keep the tube on ice until required.

3. Familiarise yourself with the PromethION Flow Cell: **inlet port cover**, **waste channel**, **waste port**.

※ **Keep the flow cell inserted in the sequencing device during the whole wash** (secured, temperature controlled). The light shield may stay on.



Step B. Pause/stop the run and remove the waste buffer

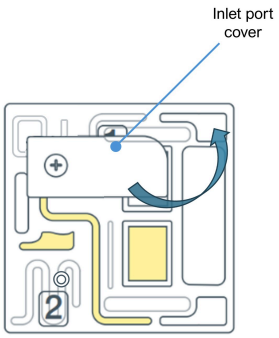
※ **Equipment :** P1000 pipette and tips

1. **Stop or pause** the sequencing experiment in MinKNOW.

※ Never manipulate the flow cell while it is sequencing – this damages the flow cell and loses pores.

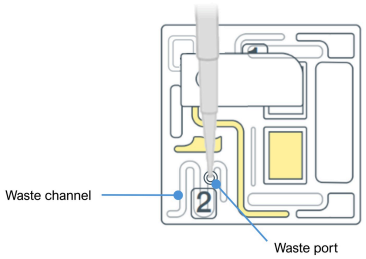
2. Ensure the flow cell **inlet port cover is closed**.

※ **Vital:** with the inlet port open, removing waste draws air across the sensor array = significant loss of sequencing channels.



3. Remove **all** of the waste buffer from the **waste port** using a P1000 pipette:
   1). Set a P1000 pipette to **1000 µl**.
   2). Insert the tip into the **waste port**.
   3). Slowly aspirate; dispense and repeat until the **waste channel is empty**.

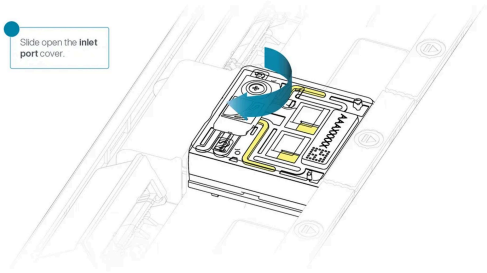
※ As the inlet port is closed, no fluid should leave the sensor array area.



Step C. Wash the flow cell

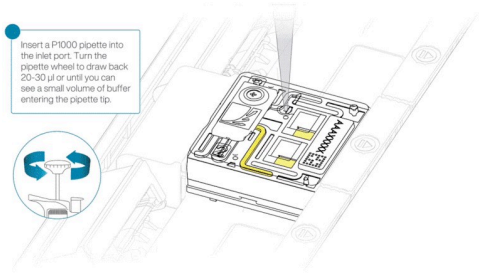
※ **Prewarm :** Prepared Flow Cell Wash Mix (Step A) on ice; P1000 / P200 pipettes and tips

1. Slide the flow cell inlet port cover **clockwise** to open.

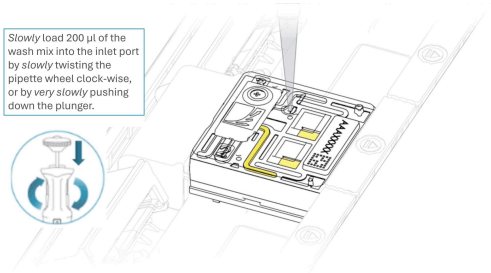


※ **Do not draw back more than 20-30 µl, and keep the array of pores covered by buffer at all times.** Air bubbles in the array irreversibly damage pores.

2. Check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:
   1). Set a P1000 pipette to **200 µl** and insert the tip into the inlet port.
   2). Turn the wheel until the dial shows **220-230 µl**, or until a small volume of buffer enters the tip.
   3). Visually check that there is continuous buffer from the inlet port across the sensor array.



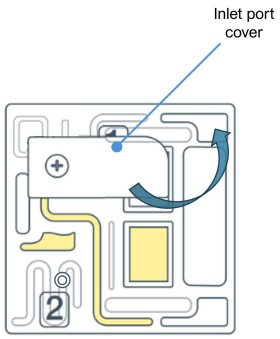
3. **Slowly** load **200 µl** of the Flow Cell Wash Mix into the inlet port with a P1000 (no bubbles in the tip). Twist the pipette wheel down (or push the plunger very slowly), leaving a small volume in the tip; dispensing should take **at least 5 seconds** at a continuous rate.



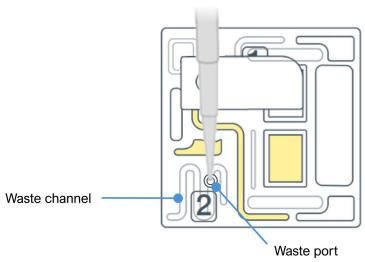
4. Set a timer and incubate the wash mix on the flow cell for **5 minutes**.

5. Repeat step 3: load the **second 200 µl** of the Flow Cell Wash Mix.

6. **Close the inlet port** and wait for **1 hour**.



7. Remove **all** of the waste buffer from the **waste port** using a P1000 pipette (inlet port closed; as Step B.3).



※ The flow cell is now washed. Go to **Step D** (store the flow cell – the usual route) or **Step E** (load a new library).

※ **Do not store the flow cell with the wash mix on the array** – irreversible pore loss.

Step D. Option 1 : Store the flow cell for later use

※ **Prewarm :** Storage Buffer (S) – thaw one tube at RT; P1000 pipette and tips

1. Slide the inlet port cover clockwise to open the inlet port.
2. Check for an air bubble under the cover and draw back **20-30 µl only** (P1000 set to 200 µl, turn to 220-230 µl; as Step C.2). Check for continuous buffer across the sensor array.
3. **Slowly** load **500 µl** of Storage Buffer (S) into the inlet port with a P1000 (no bubbles in the tip). Twist the pipette wheel down (or push the plunger very slowly), leaving a small volume in the tip; dispensing should take **at least 5 seconds** at a continuous rate.
4. **Close the inlet port**, then remove **all** of the waste buffer from the **waste port** with a P1000 (as Step B.3).
5. Store the flow cell at **2–8°C**.

※ To re-use: take the flow cell from storage and let it warm to room temperature for **5 minutes**.
※ After a wash or storage, run a **Flow cell check** in MinKNOW with the Storage Buffer (S) still on the flow cell to count the active pores; then prime the flow cell and load the library (Step E).

Step E. Option 2 : Run a second library on the flow cell

※ **Prewarm :** Kit 14 reagents (sequencing kit, EXP-AUX003 or EXP-FLP004)
a. Sequencing Buffer (SB)
b. Library Beads (LIB) or Library Solution (LIS)
c. Flow Cell Tether (FCT)
d. Flow Cell Flush (FCF)
thaw at RT, vortex, spin down, store on ice

※ These buffers are **incompatible with a Flow Cell Check** before loading; the first pore scan of the new run reports the available pores.

1. Prepare the flow cell priming mix and mix thoroughly by pipetting:

Reagent	**Volume per flow cell**
Flow Cell Flush (FCF)	**1,170 µl**
Flow Cell Tether (FCT)	**30 µl**
Total volume	**1,200 µl**

2. Slide the inlet port cover clockwise to open. Check for an air bubble under the cover and draw back **20-30 µl only** (P1000 set to 200 µl, turn to 220-230 µl; as Step C.2).

3. **Slowly** load **500 µl** of the priming mix into the inlet port. Twist the pipette wheel down (or push the plunger very slowly), leaving a small volume in the tip; dispensing should take **at least 5 seconds** at a continuous rate.

4. Close the inlet port and wait **5 minutes**. Meanwhile prepare the library (steps 5-6).
※ **It is vital to wait five minutes between the priming mix flushes to remove the nuclease.**

5. Thoroughly mix the Library Beads (LIB) by pipetting **immediately before use** (beads settle very quickly). Use LIS instead for viscous libraries.

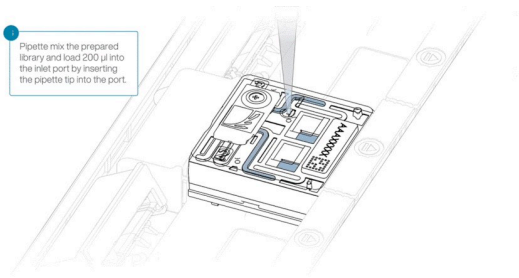
6. In a new tube, prepare the library for loading (check the volumes in the protocol of your sequencing kit):

Reagent	**Volume per flow cell**
Sequencing Buffer (SB)	**100 µl**
Library Beads (LIB), mixed before use, or LIS	**68 µl**
DNA library	**32 µl**
Total volume	**200 µl**

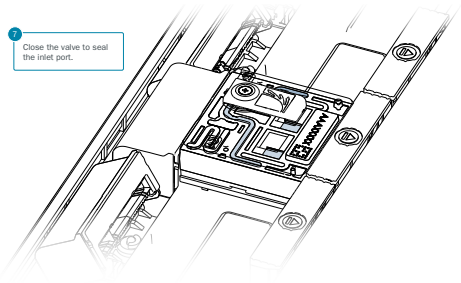
7. Remove **all** of the waste buffer from the **waste port** with a P1000 (**inlet port closed**; as Step B.3).

8. Open the inlet port and **slowly** load the **second 500 µl** of the priming mix (at least 5 seconds).

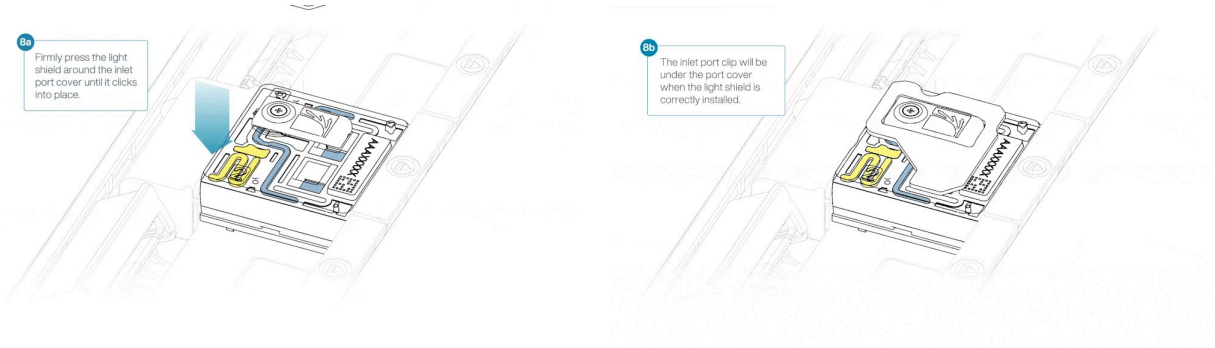
9. Mix the prepared library gently by pipetting just prior to loading. **Slowly** load **200 µl** of library into the inlet port with a P1000 (at least 5 seconds, continuous rate).



10. Close the valve to seal the inlet port.



11. **Install the light shield as soon as the library is loaded.** Align the inlet-port cut-out with the inlet port cover (leading edge above the flow cell ID), then press firmly around the inlet port cover until the clip clicks underneath it.



12. Close the PromethION lid. Wait **at least 10 minutes** after loading before starting the run in MinKNOW.

※ Library storage: Eppendorf DNA LoBind tubes, **4°C** short term / re-loading between washes; **-80°C** for single use and > 3 months.