SOP

10.3.7 Washing a flow cell (Oxford Nanopore Technology)

Commenté [1]: It is important to check the version of the protocols on the ONT website regurarly. They change and update them without informing the customers.
This version is based on ONT document version WFC_9120_v1_revS_25Jul2025

Objectives and scope

This SOP describes the various steps to follow to wash the Nanopore Flow Cell for sequencing (i.e. the MinION flow cell) before being reused for sequential runs.

This SOP is intended for Mini-Lab laboratory technicians.

Principle

This procedure of washing a flow cell is key as it provides the opportunity to store and re-utilise the same flow cell several times, maximising the available run time.

The Flow Cell Wash Kit works by flushing out and digesting the first library; refreshing the system allows for the cell to be stored safely and a subsequent library to be loaded. The nuclease digests the DNA library. After, removing the waste buffer from the waste ports after each flush step is highly recommended. This is to ensure there is no nuclease in the waste channel that may diffuse through the flow cell during sequencing.
Following the wash step, Storage Buffer can be introduced into the flow cell, allowing storage of the flow cell at 4-8°C.

- This protocol assumes that the flow cell has already had a DNA/RNA library run on it.
- The aim is to remove most of this initial library from the flow cell
- The Wash Kit contains all solutions required for removal of the initial library
- Data acquisition in MinKNOW should be stopped (if loading a new library or storing the flow cell), or paused (if loading more of the same library after the wash)
- After the flow cell has been washed, the flow cell can be stored at 4°C
- This version is based on ONT document version WFC_9120_v1_revS_25Jul2025

Safety and environment

- Wear your PPE for the duration of this technique: lab coat, gloves;
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

Flow cells are irreversibly damaged when frozen!

All flow cells should be stored at 2-8°C from receipt to ensure optimal performance.

Sample

- Type of material:
    - A flow cell for sequencing and various buffers/solutions

Equipment

Common Name	*Associated SOP*
Refrigerator 4-8°C	SOP 7.7 REFCON
Freezer -20°C	SOP 7.7 REFCON
Nanopore Flow Cell MIN114 R10	SOP TBD
Vortex	SOP TBD
Minispinner	SOP TBD

Consumables

*Common Name**	*Storage conditions*
1.5 or 2 ml Eppendorf DNA LoBind tubes	*N/A*
Flow Cell Wash Kit (EXP-WSH004)	*2-25°C*
P1000 pipette and tips	*N/A*
P20 pipette and tips	*N/A*
P10 pipette and tips	*N/A*
Bucket with ice	*N/A*

Flow Cell Wash Kit Contents

Name	*Acronym*	*Volume (µL)*	*N of vials*	*N of uses*	*Storage conditions*
Wash Mix	**WMX**	15	1	6	*-20°C*
Wash Diluent	**DIL**	1300	2	6	*-20°C*
Storage Buffer	**S**	1600	2	6	*-20°C*

- Wash Mix (WMX) contains DNase I.
- Wash Diluent (DIL) contains the exonuclease buffer to maximise activity of the DNase I.
- The Storage Buffer allows flow cells to be stored for extended period of time.

Flushing a MinION Flow Cell

Before starting

IMPORTANT
A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.

1. Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.

2. Thaw one tube of Wash Diluent (DIL) at room temperature
3. Mix the contents of Wash Diluent (DIL) thoroughly by vertexing, then spin down briefly and place on ice.
4. In a fresh 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:

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

5. Mix well by pipetting and place on ice. Do NOT VORTEX the tube. **Store the prepared Flow Cell Wash Mix tube on ice until required.**
6. Pause/stop the sequencing run and remove the waste buffer

IMPORTANT
Keep your flow cell on your sequencing device during the whole wash procedure.
Oxford Nanopore Technology (ONT) strongly recommends that the flow cell is kept inserted in the sequencing device to ensure the equipment is secured, minimise component damage and potential errors, and maximise usability.

7. Familiarize with the flow cell

8. **Ensure the flow cell priming port and SpotON sample port covers are closed.**

IMPORTANT
It is vital that **the flow cell priming port and SpotON sample port are closed before removing the waste buffe**r to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

9. **Remove all of the waste buffers from waste port 1 by using a P1000 pipette.**

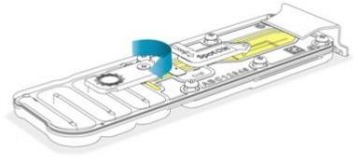
You should remove the full volume of waste buffer from the waste channel. If required, you can dispense the volume aspirated from the waste channel and repeat the process to remove the full volume from the waste channel.

- Set your P1000 pipette to 1000 µl.
- Insert the P1000 pipette tip into waste port 1.
- Slowly aspirate to remove the waste buffer from waste port 1.

Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.

Wash the flow cell

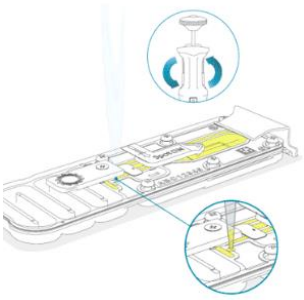
10. Slide the flow cell priming port cover clockwise to open



IMPORTANT
TAKE CARE when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times.
Introducing air bubbles into the array can irreversibly damage pores.

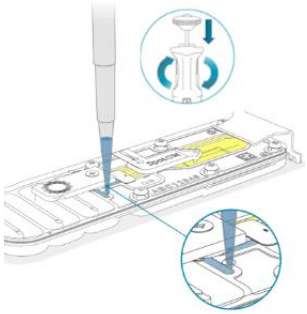
11. After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

    - Set a P1000 pipette to 200 µL
    - Insert the tip into the flow cell priming port
    - Turn the wheel (if allowed with your pipette) until the dial shows 220-230 µL or until you can see a small volume of buffer entering the tip
    - Visually check that there is continuous buffer from the flow cell priming port across the sensor array



12. **Slowly** load 200µl of the prepared flow cell wash mix into the priming port, as follows

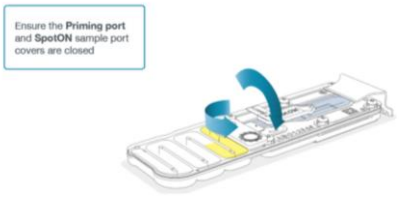
    - Using a P1000 pipette, take 200 µl of the flow cell wash mix.
    - Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
    - Slowly twist the pipette wheel down to load the flow cell (if allowed by your pipette) or push down the plunger **very slowly**, leaving a small volume of buffer in the pipette tip



    - Set a timer for a 5 minutes incubation.

13. Once the 5 minutes incubation is complete, repeat the flow cell wash mix loading step (step 12), for a second 200 µl load of the prepared wash mix

14. Gently close the priming port and wait for 1 hour.



15. After 1 hour, **remove all of the waste buffers from waste port 1 by using a P1000 pipette.**

END OF WASHING PROCEDURE

Your flow cell has now been washed, and you can proceed to

- Store your flow cell for later use

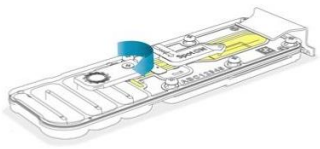
Note: Do not store your flow cell with the wash mix on the array. This can lead to irreversible pore loss.

Store the MinION flow cell for later use

16. Thaw one tube of **storage buffer (S)** at room temperature (between 18 and 23 °C).

17. Mix contents thoroughly by pipetting and spin down briefly.

18. Slide the flow cell **priming port cover** clockwise to open.



19. After opening the **priming port,** check for a small air bubble under the cover. With your pipette, slowly draw back a small volume to remove any bubbles:

- Set the P1000 pipette to 200µL.
- Insert the tip into the flow cell priming port.
- Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip

Note: Take care when drawing back buffer from the flow cell, do not remove more than 20-30 µl. Visually check that there is continuous buffer from the priming port across the sensor array.

20. Slowly load 500 µl of the Storage Buffer (S) into the priming port, as follows:
    - Using a P1000 pipette, take 500 µl of the Storage Buffer (S).
    - Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
    - Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip.

Note: Dispensing the Storage Buffer (S) into your flow cell should take at **least 5 seconds**, ensuring it is done very slowly and at a continuous rate.

21. Remove all of the waste buffers from waste port 1 by using a P1000 pipette.

Note: You should remove the full volume of waste buffer from the waste channel. If required you can dispense the volume aspirated from the waste

Commenté [2]: I would end the washing protocol here. If they want to re-use the flowcell for a next run they have to use the SOP in which the process of library prep and loading flowcell is described

channel and repeat the process to remove the full volume from the waste channel.

- Close the priming port.
- Set your P1000 pipette to 1000 µl.
- Insert the P1000 pipette tip into waste port 1.
- Slowly aspirate to remove the waste buffer from waste port 1.

Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.

22. Close the priming port

23. **The flow cell can now be stored at 2–8°C.**

When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to room temperature for ~5 minutes.

Note: After performing a flow cell wash or storing your flow cell, Oxford Nanopore recommends using running a 'Flow cell check' to check number of available nanopores.

- Load your flow cell into the device with Storage Buffer (S) and start a Flow cell check to detect the number of active pores. For more information, please visit the Flow cell check section of the MinKNOW protocol.
- After the Flow cell check, prime your flow cell and load the library before starting a new sequencing run.

Library storage recommendations
ONT recommends storing libraries in Eppendorf DNA LoBind tubes at 4°C for short term storage or repeated use, for example, reloading flow cells between washes. For single use and long-term storage of more than 3 months, we recommend storing libraries at -80°C in Eppendorf DNA LoBind tubes. For further information, please refer to the DNA library stability Know-How document.

Related documents

- Commercial SOP Flow Cell Wash Kit. Oxford Nanopore Technologies. https://nanoporetech.com/document/flow-cell-wash-kit-exp-wsh004

IMPORTANT: verify the latest version of the protocol published on the ONT website!

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- Priming video: https://community.nanoporetech.com/nanopore_learning/lessons/priming-and-loading-your-flow-cell
- SOP-10.3.3. DNA extraction for sequencing
- SOP 10.3.4. DNA quantification using Qubit
- SOP-10.3.5: Rapid Sequencing gDNA barcoding and library preparation
- SOP 10.3.6. Oxford Nanopore Sequencing – priming and loading a flow cell
- DOC-6.8-DECHINT: 6.8 Internal waste management
- Commercial SOP: Rapid sequencing gDNA -barcoding (SQK-RBK110.96)