MinION

Flow Cell Wash Kit (EXP-WSH004 or EXP-WSH004-XL)

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Kit batch number ..... **Flow cell number** ..... **DNA Samples** .....

Checklist: Method considerations and identifying your MinION and GridION Flow Cell components

Materials	Consumables	Equipment
Method considerations and identifying your MinION and GridION Flow Cell components		**Notes / Observations**

Keep your flow cell on your sequencing device during the whole wash procedure.

We strongly recommend your flow cell is kept inserted in the sequencing device to ensure the equipment is secured and to maintain proper temperature control throughout the wash procedure.

1 Familiarise yourself with the MinION and GridION Flow Cell components.

If required, perform library recovery from your flow cell(s).

If you are planning on recovering your current sequencing library from your flow cell before proceeding with the flow cell wash, **please ensure this is the first thing you do**.

Guidance on how to recover your library from your flow cell can be found in our library recovery from flow cells protocol.

2 Watch the washing and reloading a MinION and GridION Flow Cell video.

This video will show you how to wash a flow cell after a sequencing run and how to load a new library. We strongly recommend all our users to view the demonstration video before performing this method:

Washing and reloading a MinION and GridION Flow Cell video

Checklist: Prepare your Flow Cell Wash Kit reagents

Materials	Consumables	Equipment
☐ Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)	☐ 1.5 ml Eppendorf DNA LoBind tubes	☐ P1000 pipette and tips ☐ P20 pipette and tips ☐ Vortex mixer ☐ Microfuge ☐ Ice bucket with ice

Prepare your Flow Cell Wash Kit reagents

We strongly recommend preparing your flow cell wash reagents fresh for each use.

Do not pre-prepare your Flow Cell Wash Mix and store it for >1 day, as this could result in sub-optimal results.

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 vortexing, 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:

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

Mix well by pipetting, and place the tube(s) on ice.

Note: Do not vortex the Flow Cell Wash Mix tube.

Store the prepared Flow Cell Wash Mix tube on ice until required.

Notes / Observations

Checklist: Pause/stop the sequencing run and remove the waste buffer

Materials	Consumables	Equipment
		☐ P1000 pipette and tips

Pause/stop the sequencing run and remove the waste buffer	Notes / Observations

Keep your flow cell on your sequencing device during the whole wash procedure.

We strongly recommend your flow cell is kept inserted in the sequencing device to ensure the equipment is secured, minimise component damage and potential errors, and maximise usability.

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

Please ensure your flow cell is not sequencing while performing any of the wash steps. Manipulating the flow cell while it is sequencing can results in damage and pore loss.

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

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

3 **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.

1. Set your P1000 pipette to 1000 µl.
2. Insert the P1000 pipette tip into waste port 1.
3. 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.

Proceed to wash your flow cell.

Checklist: Wash the flow cell

Materials	Consumables	Equipment
☐		☐ P1000 pipette and tips ☐ P200 pipette and tips ☐ Ice bucket with ice

Wash the flow cell	Notes / Observations

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

> **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.**

2. After opening the priming port, 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.
    2. Insert the tip into the flow cell priming port.
    3. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip.
    4. Visually check that there is continuous buffer from the flow cell priming port across the sensor array.

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

    1. Using a P1000 pipette, take 200 µl of the flow cell wash mix
    2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
    3. 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 wash mix into your flow cell should take at **least 5 seconds**, ensuring it is done very slowly and at a continuous rate.

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

5. Once the 5 minute incubation is complete, repeat the flow cell wash mix loading step (step 3), for a second 200 µl load of the prepared wash mix.
6. Close the priming port and wait for 1 hour.
7. Remove all of the waste buffers from waste port 1 by using a P1000 pipette.

Your flow cell has now been washed and you can proceed to the next step.

Proceed to:

- Option 1: Run a second sequencing library on your flow cell
- Option 2: 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.

Checklist: Option 1: Run a second sequencing library on your flow cell

Materials	Consumables	Equipment
☐ Flow cell priming reagents available in your sequencing kit or in the following kits: ☐ Sequencing Auxiliary Vials V14 (EXP-AUX003) ☐ Flow Cell Priming Kit V14 (EXP-FLP004)		☐ P1000 pipette and tips ☐ P20 pipette and tips ☐ Ice bucket with ice

Option 1: Run a second sequencing library on your flow cell

Notes / Observations

The sequencing reagents outlined in this method are for our most recent V14 chemisty.

If using a previous version of our chemistry or a kit with specific sequencing reagents, please ensure you are using the correct sequencing reagents for your application.

The buffers used in this process are incompatible with conducting a Flow Cell Check prior to loading a subsequent library. However, the first pore scan once a sequencing run has started will report the number of nanopores available.

1. Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at room temperature before mixing by vortexing. Then spin down and store on ice.

For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.

Note: We do not recommend using any other albumin type (e.g. recombinant human serum albumin).

2. Prepare the flow cell priming mix with BSA by combining the following reagents and thoroughly mixing by pipetteing:

Reagent	Volume per flow cell
Flow Cell Flush (FCF)	1,170 µl
Bovine Serum Albumin (BSA) at 50 mg/ml	5 µl

Reagent	Volume per flow cell
Flow Cell Tether (FCT)	30 µl
Total volume	**1,205 µl**

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

4. After opening the priming port, 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
    2. Insert the tip into the priming port
    3. 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.

5. Slowly load 800 µl of the priming mix into the priming port, as follows:

    1. Using a P1000 pipette, take 800 µl of the priming mix.
    2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip.
    3. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, as illustrated in the demonstration video, leaving a small volume of buffer in the pipette tip.

Note: Dispensing the 800 µl of priming mix into your flow cell should take at least 10 seconds, ensuring it is done very slowly and at a continuous rate.

> **It is vital to wait five minutes between the priming mix flushes to effectively remove the nuclease.**

6. Close the priming port and wait five minutes.

During this time, prepare the library for loading by following the steps below.

7. Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

We recommend using the Library Beads (LIB) for most sequencing experiments. However, the Library Solution (LIS) is available for more viscous libraries.

8 In a new tube, prepare the library for loading according to the "Priming and loading the MinION and GridION Flow Cell" section of the suitable protocol to ensure you are using the correct reagents and volumes.

Reagent	Volume per flow cell
Sequencing Buffer (SB)	37.5 µl
Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using	25.5 µl
DNA library	12 µl
Total	**75 µl**

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.

1. Set your P1000 pipette to 1000 µl.
2. Insert the P1000 pipette tip into waste port 1.
3. 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.

10 Slowly load 200 µl of the priming mix into the flow cell priming port, as follows:

1. Open the priming port and gently lift open the SpotON sample port.
2. Using a P1000 pipette, take 200 µl of the priming mix.
3. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip.
4. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very**

slowly, as illustrated in the demonstration video, leaving a small volume of buffer in the pipette tip.

Note: Dispensing the 200 µl of priming mix into your flow cell should take at least 5 seconds, ensuring it is done very slowly and at a continuous rate.

11 Mix the prepared sequencing library gently by pipetting up and down just prior to loading.

12 Add 75 µl of the prepared sequencing library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.

13 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.

Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.

We recommend leaving the light shield on the flow cell when library is loaded, including during any washing and reloading steps. The shield can be removed when the library has been removed from the flow cell.

14 Place the light shield onto the flow cell, as follows:

1. Carefully place the leading edge of the light shield against the clip. **Note:** Do not force the light shield underneath the clip.

2. Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.

The MinION Flow Cell Light Shield is not secured to the flow cell and careful handling is required after installation.

Close the device lid and continue sequencing run on MinKNOW.

Checklist: Option 2: Store your flow cell for later use

Materials	Consumables	Equipment
☐ Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)		☐ P1000 pipette and tips ☐ P20 pipette and tips

Option 2: Store your flow cell for later use

Notes / Observations

1. **Thaw one tube of Storage Buffer (S) at room temperature.**
2. **Slide the flow cell priming port cover clockwise to open.**
3. **After opening the priming port, 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
    2. Insert the tip into the priming port
    3. 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.

4. **Slowly load 500 µl of the Storage Buffer (S) into the priming port, as follows:**

    1. Using a P1000 pipette, take 500 µl of the Storage Buffer (S).
    2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
    3. 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.

5. **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.

1. Close the priming port.
2. Set your P1000 pipette to 1000 µl.
3. Insert the P1000 pipette tip into waste port 1.
4. 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.

6 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.

After performing a flow cell wash or storing your flow cell, we recommend 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 our MinKNOW protocol.

After the Flow cell check, prime your flow cell and load the library before starting a new sequencing run.