Flow Cell Wash Kit (EXP-WSH004)

Version: WFC_9120_v1_revL_08Dec2020
Last update: 19/09/2023

Flow Cell Number: ..... DNA Samples: .....

Before start checklist

Materials

- [ ] Flow Cell Wash Kit (EXP-WSH004)
- [ ] Flow cell priming reagents available in your sequencing kit or in the following kits:
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003) or Sequencing Auxiliary Vials (EXP-AUX002 or EXP-AUX001)
- [ ] Flow Cell Priming Kit (EXP-FLP004) or Flow Cell Priming Kit (EXP-FLP002)

Consumables

Equipment

- [ ] Ice bucket with ice
- [ ] Pipettes and pipette tips P20, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Flushing a MinION/GridION Flow Cell	

Preparation to run the washing procedure

- [ ] Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.
- [ ] Thaw one tube of Wash Diluent (DIL) at RT.
- [ ] Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.

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

- [ ] 2 µl Wash Mix (WMX)
- [ ] 398 µl Wash Diluent (DIL)

- [ ] Mix well by pipetting, and place on ice. Do not vortex the tube.
- [ ] Stop or pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.
- [ ] Before removing the waste fluid, ensure that the flow cell priming port cover and SpotON sample port cover are closed, as indicated in the figure below.

IMPORTANT

- [ ] It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

- [ ] Using a P1000, remove all fluid from the waste channel through waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.
- [ ] Rotate the flow cell priming port cover clockwise so that the priming port is visible.

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.

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INSTRUCTIONS	NOTES/OBSERVATIONS
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 until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip. ☐ Visually check that there is continuous buffer from the flow cell priming port across the sensor array. ☐ Load 400 µl of the prepared Flow Cell Wash Mix into the flow cell priming port, avoiding the introduction of air. ☐ Close the flow cell priming port and wait for 60 minutes. ☐ Before removing the waste fluid a second time, ensure that the flow cell priming port cover and SpotON sample port cover are closed, as indicated in the figure below.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
☐ Using a P1000, remove all fluid from the waste channel through waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.	
Follow one of the two options described in the next steps of the protocol.	
To run a second library on a MinION/GridION flow cell straight away	
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	
To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of your library preparation protocol with the recommendations below. ☐ Pipette very slowly when loading priming mix into the flow cell. ☐ Wait five minutes between priming mix flushes. ☐ After the five minute pause, close the priming port, ensure the SpotON port is closed and remove the waste from waste port 1. This prevents the nuclease from diffusing through the flow cell. Repeat this step after the second priming mix flush.	
IMPORTANT ☐ When priming a flow cell after a nuclease wash with the Flow Cell Wash Kit, it is vital to wait five minutes between the priming mix flushes and to remove the waste for effective removal of the nuclease.	
To store the MinION/GridION flow cell for later use	
Storage Buffer (S) can be used to flush flow cells for storage for later use or to check number of available nanopores before loading another library. ☐ Thaw one tube of Storage Buffer (S) at RT. ☐ Mix contents thoroughly by pipetting and spin down briefly.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Rotate the flow cell priming port cover clockwise so that the priming port is visible. 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 until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip. ☐ Visually check that there is continuous buffer from the flow cell priming port across the sensor array. ☐ Slowly add 500 µl of Storage Buffer (S) through the flow cell priming port. ☐ Close the priming port. ☐ Using a P1000, remove all fluid from the waste channel through waste port 1. As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
☐ The flow cell can now be stored at 4-8°C.	
When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to RT for ~5 minutes.	
IMPORTANT After performing a flow cell wash or storing your flow cell, we recommend using the first pore scan to check number of available nanopores. ☐ stop your sequencing run, prime your flow cell and load the library before starting a new sequencing run. ☐ or, pause your sequencing run, prime your flow cell and load the library before restarting the sequencing run.	