Flow Cell Wash Kit (EXP-WSH003)

Version: WFC_9088_v1_revE_18Sep2019
Last update: 12/03/2020

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

Before start checklist

Materials

- [ ] Flow Cell Wash Kit (EXP-WSH003)

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 Solution A on ice. Do not vortex the tube. ☐ Thaw one tube of Wash Solution B at RT. ☐ Mix the contents of Wash Solution B thoroughly by vortexing, spin down briefly and place on ice. In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Wash Mix: ☐ 20 µl Wash Solution A (A) ☐ 380 µl Wash Solution B (B) ☐ 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. ☐ Ensure that the priming port cover and SpotON sample port cover are in the positions indicated in the figure below. ☐ Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the 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.	
☐ Rotate the flow cell priming port cover clockwise so that the priming port is visible. Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few µls): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the 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 priming port across the sensor array.	
IMPORTANT ☐ Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µls, 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
☐ Load 400 µl of the prepared Wash Mix into the flow cell via the priming port, avoiding the introduction of air. ☐ Close the priming port and wait for 30 minutes. ☐ Ensure that the priming port cover and SpotON sample port cover are in the positions indicated in the figure below. ☐ Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the 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.	
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 ☐ The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading the subsequent library. In order to check your flow cell, follow the instructions in the next section “To store the MinION/GridION/PromethION flow cell for later use” before priming and loading the flow cell.	
☐ To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of the relevant protocol.	
To store the MinION/GridION flow cell for later use	
☐ Thaw one tube of Storage Buffer (S) at RT. ☐ Mix contents thoroughly by pipetting and spin down briefly. ☐ Rotate the flow cell priming port cover clockwise so that the priming port is visible. Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few µls): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the 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 priming port across the sensor array. ☐ Slowly add 500 µl of Storage Buffer (S) through the priming port of the flow cell. ☐ Close the priming port. ☐ Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
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.	
Flushing a PromethION flow cell	
Preparation to run the washing procedure. ☐ Place the tube of Wash Solution A on ice. Do not vortex the tube. ☐ Thaw one tube of Wash Solution B at RT. ☐ Mix the contents of Wash Solution B thoroughly by vortexing, spin down briefly and place on ice. In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Wash Mix: ☐ 20 µl Wash Solution A (A) ☐ 380 µl Wash Solution B (B) ☐ 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. ☐ Rotate the inlet port cover clockwise to reveal the inlet port. A small tract of air may be visible beyond the inlet port. If necessary, using a P1000 draw back a small volume to remove any air (a few µls): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the inlet 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. ☐ Load 400 µl of the prepared Wash Mix into the flow cell via the inlet port, avoiding the introduction of air. ☐ Close the inlet port and wait for 30 minutes.	
Follow one of the two options described in the next steps of the protocol.	
To run a second library on a PromethION flow cell straight away	
IMPORTANT ☐ The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading the subsequent library. In order to check your flow cell, follow the instructions in the next section “To store the MinION/GridION/PromethION flow cell for later use” before priming and loading the flow cell.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of the relevant protocol.	
To store the PromethION flow cell for later use	
☐ Thaw one tube of Storage Buffer (S) at RT. ☐ Mix contents thoroughly by pipetting and spin down briefly. ☐ Rotate the inlet port cover clockwise to reveal the inlet port. A small tract of air may be visible beyond the inlet port. If necessary, using a P1000 draw back a small volume to remove any air (a few µls): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the inlet 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. ☐ Slowly add 500 µl of Storage Buffer through the inlet port of the flow cell. ☐ Close the inlet port cover, and remove any buffer from the waste port at the top of the flow cell. ☐ The flow cell can now be stored at 4-8° C. ☐ When you wish to reuse the flow cell, remove from storage, and allow it to warm to RT for ~5 minutes.	