

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

Version: WFC_9120_v1_revP_08Dec2020
Last update: 02/04/2024



Flow Cell Number:

DNA Samples:

Before start checklist

Materials

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

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

IMPORTANT

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

IMPORTANT

- ☐ 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.
- Remove the waste buffer, as follows:
- ☐ Close the priming port and SpotON sample port cover, as indicated in the figure below.
 - ☐ Insert a P1000 pipette into waste port 1 and remove the waste buffer.
 - ☐ Slide the flow cell priming port cover clockwise to open.

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<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette to 200 µl. <input type="checkbox"/> Insert the tip into the flow cell priming port. <input type="checkbox"/> 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. <input type="checkbox"/> Visually check that there is continuous buffer from the flow cell priming port across the sensor array. <p>Slowly load 200 µl of the prepared flow cell wash mix into the priming port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Using a P1000 pipette, take 200 µl of the flow cell wash mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> 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. <input type="checkbox"/> Set a timer for a 5 minute incubation. <p>Once the 5 minute incubation is complete, carefully load the remaining 200 µl of the prepared flow cell wash mix into the priming port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Using a P1000 pipette, take the remaining 200 µl of the flow cell wash mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> 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. <p><input type="checkbox"/> Close the priming port and wait for 1 hour.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>Remove the waste buffer, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Ensure the priming port and SpotON sample port covers are closed, as indicated in the figure below. <input type="checkbox"/> Insert a P1000 pipette into waste port 1 and remove the waste buffer. 	
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	
<p>IMPORTANT</p> <p><input type="checkbox"/> The sequencing reagents outlined in this method are for our most recent V14 chemistry.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.</p>	

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<input type="checkbox"/> Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT before mixing by vortexing. Then spin down and store on ice.	
IMPORTANT <input type="checkbox"/> 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.	
To prepare the flow cell priming mix with BSA, combine Flow Cell Flush (FCF) and Flow Cell Tether (FCT), as directed below. Mix by pipetting at RT. <ul style="list-style-type: none"> <input type="checkbox"/> 1,170 µl Flow Cell Flush (FCF) <input type="checkbox"/> 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml <input type="checkbox"/> 30 µl Flow Cell Tether (FCT) <input type="checkbox"/> Slide the flow cell priming port cover clockwise to open the priming port.	
IMPORTANT <input type="checkbox"/> 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 is covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.	
After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles: <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette to 200 µl <input type="checkbox"/> Insert the tip into the priming port <input type="checkbox"/> 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 <p>Note: Visually check that there is continuous buffer from the priming port across the sensor array.</p> <p>Slowly load 800 µl of the priming mix into the priming port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Using a P1000 pipette, take 800 µl of the priming mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> 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 video above, leaving a small volume of buffer in the pipette tip. 	
IMPORTANT <input type="checkbox"/> It is vital to wait five minutes between the priming mix flushes to effectively remove the nuclease.	
<input type="checkbox"/> Close the priming port and wait five minutes. <input type="checkbox"/> Thoroughly mix the contents of the Library Beads (LIB) by pipetting.	
IMPORTANT <input type="checkbox"/> 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.	
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. <ul style="list-style-type: none"> <input type="checkbox"/> 37.5 µl Sequencing Buffer (SB) <input type="checkbox"/> 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using <input type="checkbox"/> 12 µl Recovered DNA library 	

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<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>Remove the waste buffer, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Ensure the priming port and SpotON sample port covers are closed, as indicated in the figure below. <input type="checkbox"/> Insert a P1000 pipette into waste port 1 and remove the waste buffer. <input type="checkbox"/> Slide the flow cell priming port cover clockwise to open. 	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette to 200 µl <input type="checkbox"/> Insert the tip into the priming port <input type="checkbox"/> 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 <p>Note: Visually check that there is continuous buffer from the priming port across the sensor array.</p> <p>Slowly load 200 µl of the priming mix into the flow cell priming port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Ensure the priming port is open and gently lift open the SpotON sample port. <input type="checkbox"/> Using a P1000 pipette, take 200 µl of the priming mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> 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 video above, leaving a small volume of buffer in the pipette tip. 	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>Remove the waste buffer, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Close the priming port and SpotON sample port cover, as indicated in the figure below. <input type="checkbox"/> Insert a P1000 pipette into waste port 1 and remove the waste buffer. <input type="checkbox"/> Slide open the priming port cover and gently lift open the SpotON sample port cover. <input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading. <input type="checkbox"/> Add 75 µl of the prepared 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. <input type="checkbox"/> Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port. 	

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<p>IMPORTANT</p> <p><input type="checkbox"/> Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.</p> <p>Place the light shield onto the flow cell, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Carefully place the leading edge of the light shield against the clip. Note: Do not force the light shield underneath the clip. <input type="checkbox"/> 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. 	
<p>Close the device lid and continue sequencing run on MinkNOW.</p>	
<p>To store the MinION/GridION Flow Cell for later use</p> <p>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.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Thaw one tube of Storage Buffer (S) at RT. <input type="checkbox"/> Mix contents thoroughly by pipetting and spin down briefly. <input type="checkbox"/> Slide the flow cell priming port cover clockwise to open. <p>After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette to 200 µl. <input type="checkbox"/> Insert the tip into the flow cell priming port. <input type="checkbox"/> 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. <input type="checkbox"/> Visually check that there is continuous buffer from the flow cell priming port across the sensor array. <ul style="list-style-type: none"> <input type="checkbox"/> Slowly add 500 µl of Storage Buffer (S) through the flow cell priming port. <input type="checkbox"/> Close the priming port. <input type="checkbox"/> Remove all fluid from the waste channel through waste port 1 using a P1000 pipette. 	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	
<p><input type="checkbox"/> The flow cell can now be stored at 4-8°C.</p>	
<p>When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to RT for ~5 minutes.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	