

# Ligation sequencing amplicons - Native Barcoding Kit 24 V14 (SQK-NBD114.24)

Version: NBA\_9168\_v114\_revE\_15Sep2022  
Last update: 06/01/2023

Flow Cell Number: .....

DNA Samples: .....

## INSTRUCTIONS

10min, Flow cell loading 전 QC 체크 후 (20min 소요) 포어가 800개 이상일때 진행하기

### [4] Priming and loading the SpotON flow cell

#### IMPORTANT

- ☐ Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).

#### Using the Library Solution

- ☐ Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and one tube of Flow Cell Flush (FCF) at RT. Mix by vortexing and spin down.

#### IMPORTANT

- ☐ 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.

#### IMPORTANT

- ☐ We do not recommend using recombinant BSA.

To prepare the flow cell priming mix with BSA, add the following reagents directly to the tube of Flow Cell Flush (FCF), and mix by inverting the tube and pipette mix at RT:

- ☐ 5 µl **Bovine Serum Albumin (BSA) at 50 mg/ml**
- ☐ 30 µl **Flow Cell Tether (FCT)**
- ☐ 1,205 µl **Final total volume in Flow Cell Flush (FCF) tube**

- ☐ Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.

- ☐ Slide the priming port cover clockwise to open the priming port.

#### 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.

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

- ☐ Set a P1000 pipette to ~~200~~ 800 µl
- ☐ Insert the tip into the 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 820-830 µl

Note: Visually check that there is continuous buffer from the priming port across the sensor array.

- ☐ Load **800 µl of the priming mix** into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. During this time, prepare the library for loading by following the steps below.

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



매우 민감하며, 기포가 생기지 않도록 주의 하기, 열에 약하므로 샘플 로딩 후 티슈를 위에 깔고 아이스 팩 위에서 진행하기

액체가 조금 나왔다 싶으면 stop !!! 기포가 들어가면 안됩니다.

