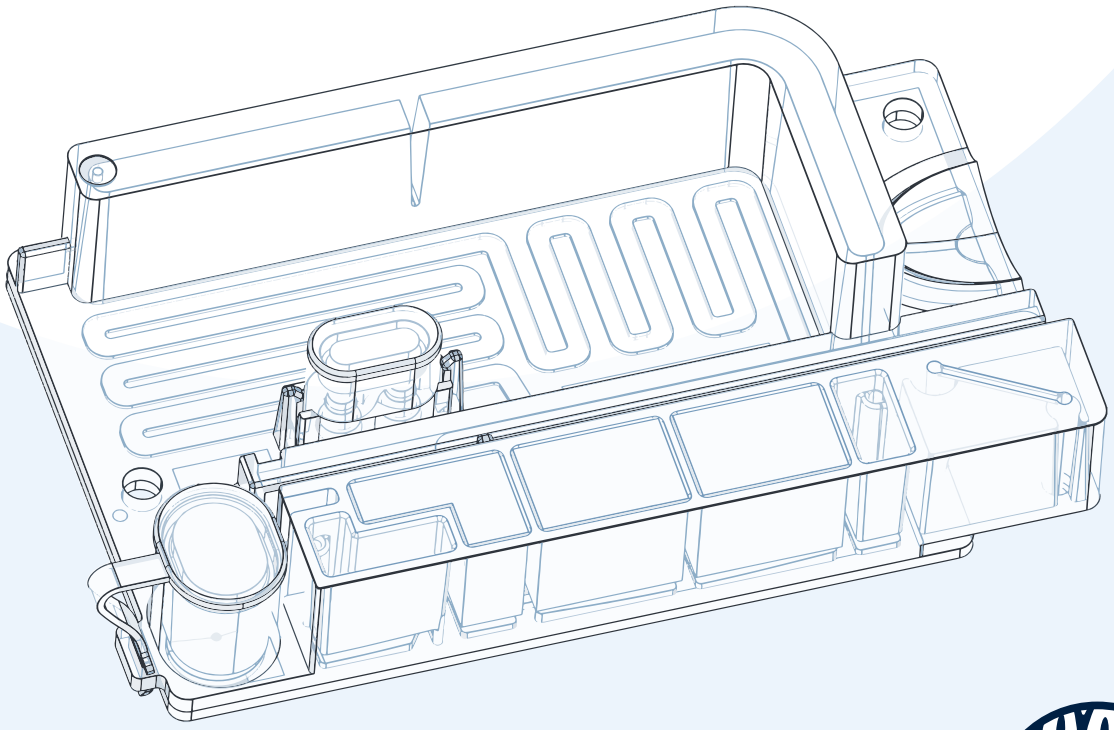
X8™ Tissue DNA Cartridge Kit

User Guide

FOR USE WITH:

X8™ OneTouch Instrument (Cat# X8-OT-101-IN)



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Manual Part Number

CGU-0010-EN *Rev C*

Legal Notices

Customer Information and Feedback

For technical information or advice, please contact our Customer Technical Support Division.

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Revision Summary

Document Number

CGU-0010-EN *Rev C*

Effective Date

September 8, 2023

Revision

- Complete Document Overhaul

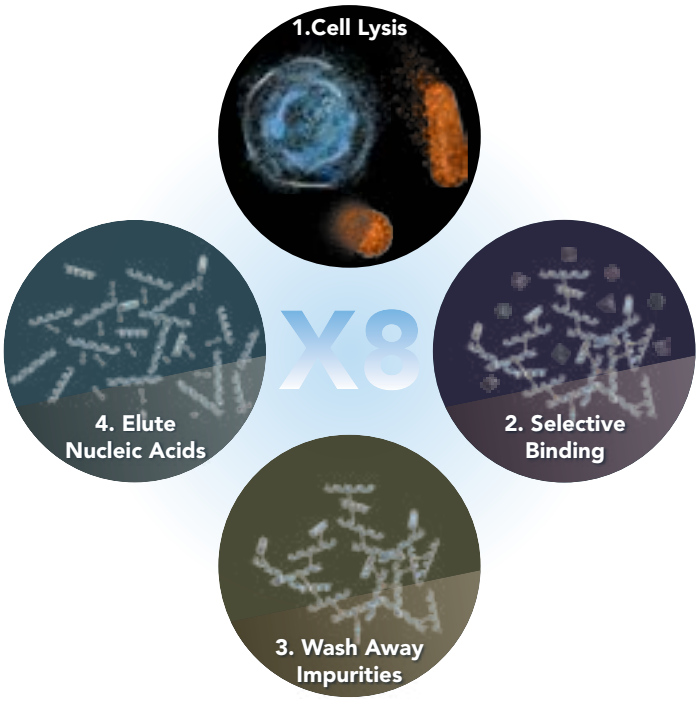
Introduction

1.1 Cartridge Kit Overview

The X8™ Tissue DNA Cartridge Kit is used with the X8™ OneTouch Instrument to provide a simple method for efficient, automated extraction of genomic DNA from fresh or frozen tissue samples.

The X8 Cartridge Kits include all necessary reagents for high-quality tissue DNA extraction. The lysis buffer has been optimized to extract genomic DNA from fresh or frozen tissue. The X8 OneTouch Instrument comes with preprogrammed, automated extraction protocols, maximizing simplicity and convenience. The touchscreen Tablet PC with the intuitive and user-friendly X8 App enables users to process up to 8 samples in less than an hour. The purified tissue DNA can then be used directly in a variety of downstream applications, including Polymerase Chain Reaction, sequencing and agarose gel electrophoresis.

Unlike typical commercial spin-column or magnetic bead technologies, the Xceler8™ Technology significantly expedites and seamlessly automates the process of gDNA extraction and purification. The novel chemistry-based approach purifies gDNA using a reversible cross-linker. It selectively binds & clusters the released gDNA from the lysed sample onto the cartridge's channel. Once impurities are washed away & sealed in the built-in waste reservoir, the purified gDNA is released and eluted using an elution buffer.



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1.2 Intended Use

The X8 Tissue DNA Cartridge Kit (Cat# X8-TD-001-24) is intended **For Research Use Only. Not for use in diagnostic procedures.** The X8 Tissue DNA Cartridge Kit has been designed for automated extraction of genomic DNA from Gram-positive bacteria and fresh or frozen tissue samples using the X8 OneTouch Instrument (Cat# X8-OT-101-IN).

1.3 Product Use Limitation

The X8 Tissue DNA Cartridge Kit (Cat# X8-TD-001-24) is not intended for the purification of RNA from tissue.

1.4 Storage of Eluted gDNA

If sample eluates are not processed immediately, store the eluted DNA at 4 °C.

Components & Storage

2.1 Cartridge Kit Components

The X8 Tissue DNA Cartridge Kit is **For Research Use Only**. **Not for use in diagnostic procedures.**

Sufficient for 24 automated extractions of genomic DNA from fresh or frozen tissue samples. Cartridges are intended for single use only.

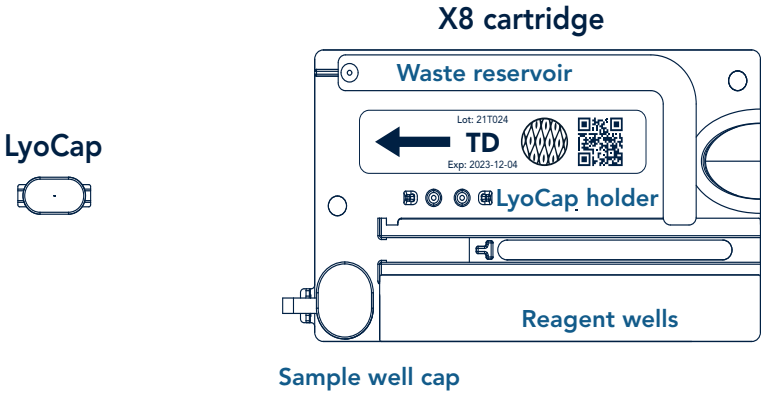
The X8 Tissue DNA Cartridge Kit includes:

- 24- X8 Tissue DNA Cartridges (PN. X8-TD02001-24)
- 9 mL- T-DNA Lysis Buffer (PN. X8-LB1)
- 5 mL- DNA Elution Buffer (PN. X8-EB1)

2.2 Cartridge Pouch Components

The X8 Tissue DNA Cartridge Kit includes:

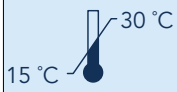
- 1- X8 Tissue DNA Cartridge (PN. X8-TD02001)
- 1- LyoCap pouch that contains a Proteinase K and cross-linker bead



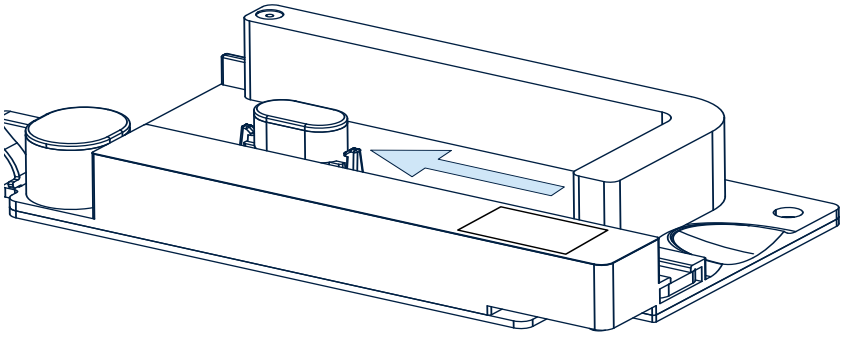
X8 Cartridge Pouch Components

2.3 Handling & Storage

In addition to the information below please follow the instructions on the X8 Cartridge Kit label for proper storage and handling of the X8 Cartridges.

SYMBOLS	DESCRIPTION
	**For single use only.** The X8 Cartridges are intended for single use only. Do not re-use.
	**For Research Use Only.** Not for use in diagnostic procedures.
	**Handling of infectious samples.** The X8 Tissue DNA Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.
	**Storage conditions.** The X8 Tissue DNA Cartridge Kits are ethanol-free and non-volatile, therefore making the X8 Cartridges suitable for storage at ambient room temperature (15-30 °C).

See figure below.	**Storage and handling orientations.** Improper shipping, storage or handling orientations may result in reagent blockage within the X8 Cartridges, which can then adversely impact the extraction performance. **Upon receiving the X8 Cartridges-** • Rest the X8 Cartridges in their proper storage orientation overnight prior to use. **Before commencing the nucleic acid extraction-** • Rest the X8 Cartridges in its proper storage orientation for a minimum of 30 min prior to use.

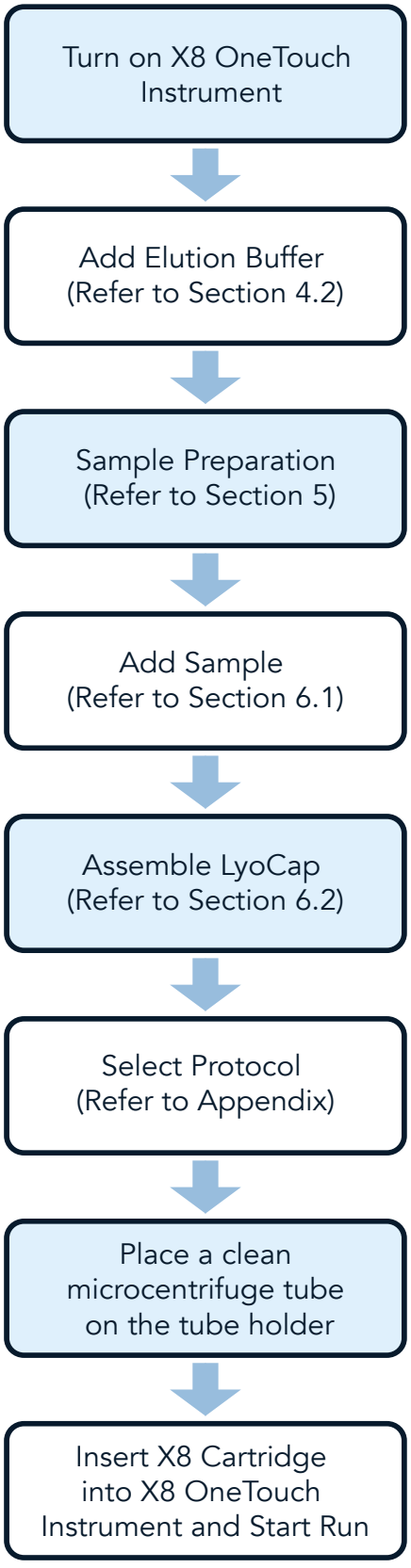


Optimal storage and handling orientation.
Set up on a flat surface with the label-side facing up.

For more information on safe handling, please refer to the Product Safety Data Sheets (SDS, CGU-0009) available at www.onebiomed.com/products.

Workflow

3.1 Workflow



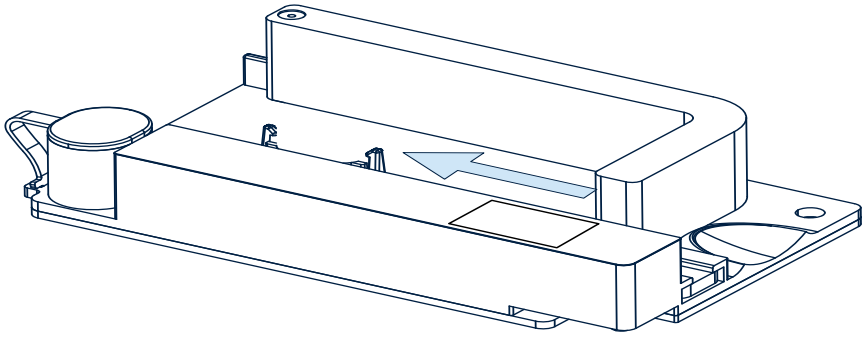
Before Each Run

4.1 Turn on X8 OneTouch

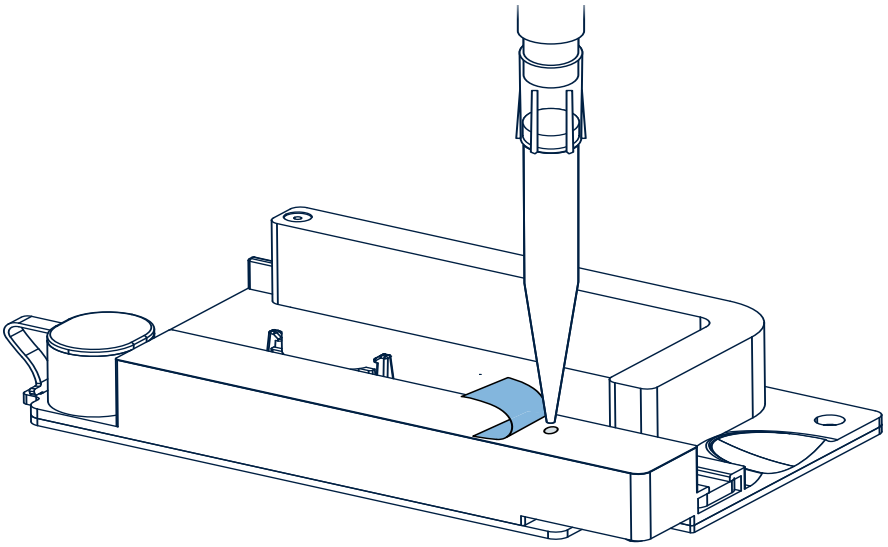
Ensure the X8 OneTouch Instrument is turned on and equilibrated. Please refer to the X8 OneTouch Operating Manual (CGU-0001) for additional details.

4.2 Elution Buffer Addition

1. Orient the X8 Cartridge on a flat surface with the reagent wells facing the user.



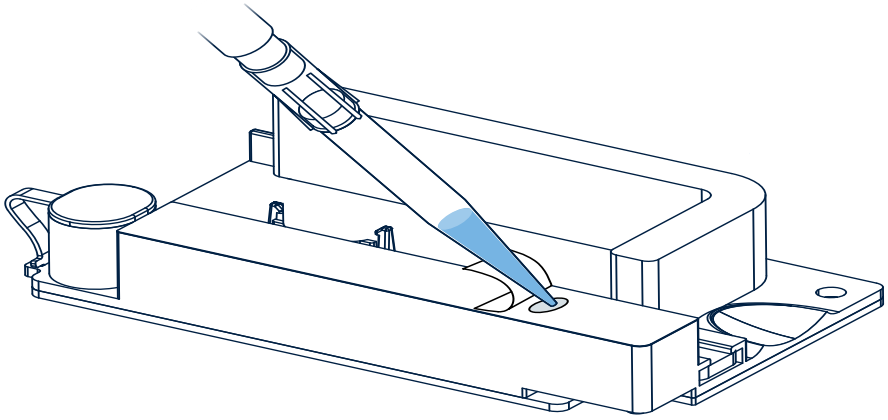
2. Gently lift the blue sticker tab on the X8 Cartridge and pierce the foil at the marked position using a sterile pipette tip.



3. Carefully aspirate 150 µL of the DNA Elution Buffer (PN. X8-EB1), ensuring there are no air bubbles.

   Note. Please refer to the appendix for additional details on varying volumes of the Elution Buffer.

4. Gently insert the pipette tip into the pierced foil until it touches the base of the well. Then position the pipette tip at a 45° angle to touch the front base of the well and then slowly dispense the DNA Elution Buffer into the well without introducing any air bubbles.

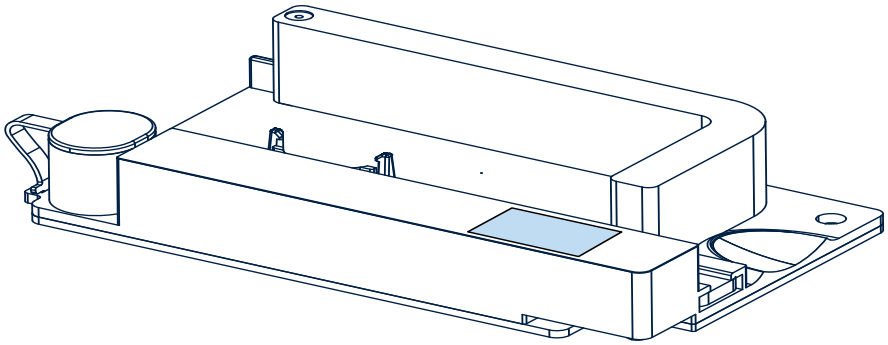


   Note. Incorrect addition of the Elution Buffer into the X8 Cartridge can result in little or no elution of the purified nucleic acid.

5. Peel the backing paper on the tape and apply the tape to seal the pierced foil. Run your finger across the sealing to ensure that it is tightly sealed.

   Note. Verify the foil is clean and dry before applying the tape. Ensure that there is no visible air gap between the pierced foil and the tape. Improper application of the tape can result in sample cap error.

   Note. Do not shake or tilt the X8 cartridge once the Elution Buffer has been added.



Sample Preparation

5.1 User Supplied Material

1. Micropipettes and sterile pipette tips.
2. Sterile microcentrifuge tubes (0.5 mL or 1.5 mL).
3. 1X PBS.
4. Vortexer.
5. Heating block for tissue dissociation.
6. Rotor-stator Homogenizer (optional).

For Gram-positive bacteria extraction (if applicable):

7. Lysozyme reconstituted as a 25 mg/mL stock in lysozyme buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA, 1.2% TritonX-100).
8. Heating block.
9. Centrifuge (with adapter for 1.5 mL / 2 mL microcentrifuge tubes).

5.2 Sample Collection & Storage

1. Best results are obtained with fresh material or material that has been immediately frozen and stored at –80 °C. Repeated freezing and thawing of stored samples should be avoided.
2. The total yield of DNA from tissue samples depends on the tissue type and mass of tissue that is processed. Each X8 Tissue DNA Cartridge is designed to purify genomic DNA from 10-30 mg of fresh or frozen tissue sample.

5.3 Rotor-stator homogenized tissue

1. Cut 10-30 mg tissue into small pieces, and place in a 1.5 mL microcentrifuge tube. We strongly recommend cutting the tissue into small pieces to enable more efficient lysis.
2. Add 125 µL of 1X PBS + 125 µL T-DNA Lysis Buffer (PN. X8-LB1) to the tissue sample.
3. Disrupt the tissue using a rotor–stator homogenizer for 20 seconds.
4. Add 1 Proteinase K bead to the tissue sample and mix thoroughly by pulse vortexing (20 times).

Note. Proceed to section 6.1 immediately after Proteinase K bead addition.

5.4 Heat block dissociated tissue

1. Cut 10 - 30 mg tissue into small pieces, and place in a 1.5 mL microcentrifuge tube. We strongly recommend cutting the tissue into small pieces to enable more efficient lysis.
2. Add 125 µL of 1X PBS and 125 µL T-DNA Lysis Buffer (PN. X8-LB1).
3. Add 1 Proteinase K bead into the tube with the tissue sample and the buffer mixture. Mix thoroughly by pulse vortexing (20 times).
4. Incubate at 56 °C for a maximum of 1 hour or until the tissue is completely lysed. Mix thoroughly by pulse vortexing (20 times).

   Note. The lysis time can be shortened to 25 min by pulse vortexing the sample mixture for 5-10 times every 5 min.

   Note. After incubation, the lysate may appear viscous. As long as the lysate can be pipetted using a 200 uL pipette tip, it can be added to the sample well of the X8 Cartridge. Otherwise, it is recommended to pulse vortex (up to 20 times) to dissociate the lysate.

   Note. Proceed to section 6.1 immediately after incubation.

5.5 Gram-Positive Bacteria

1. Obtain bacteria from an overnight culture.
2. Centrifuge to pellet the bacteria and discard supernatant. Recommended speed: 6080 x g for 5 minutes.
3. Wash the bacteria by adding at least 1 mL of PBS per 1 x 10^9 of bacteria and mix gently by pipetting to resuspend bacteria. Repeat step 2.
4. Resuspend the bacteria pellet to the final concentration of ~1 x 10^9 bacteria in 80 µL of PBS.
5. Add 120 µL of 25 mg/mL lysozyme in 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton X-100.
6. Incubate at 37 °C for 30 minutes on a heat block.
7. Add 25 µL of the T-DNA Lysis Buffer (PN. X8-LB1) and 1 Proteinase K bead into the sample mixture and mix well.

   Note. Proceed to section 6.1 immediately after T-DNA Lysis Buffer and Proteinase K addition.

5.6 Average Expected Yield by Tissue Type

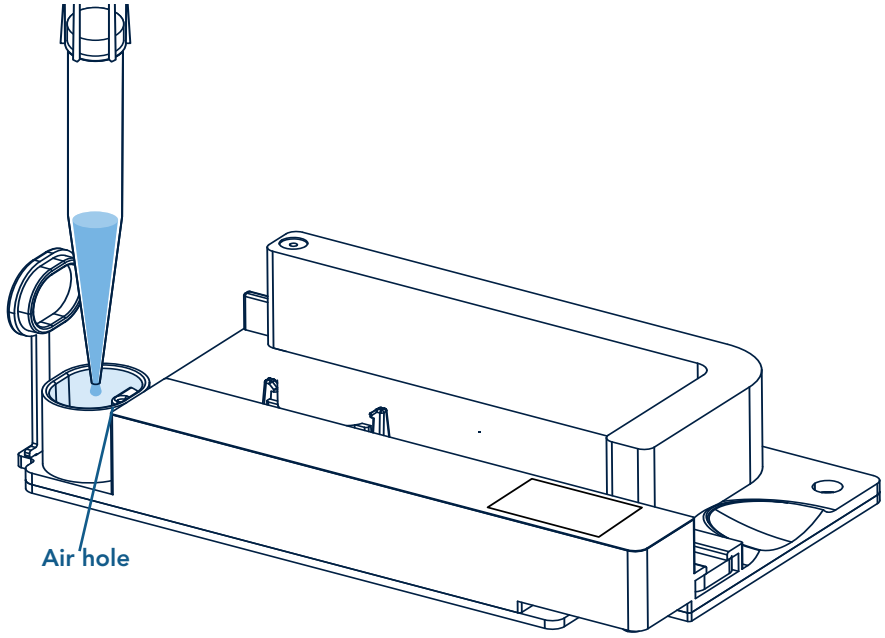
TISSUE TYPE	TISSUE INPUT (mg)	TOTAL AVERAGE DNA YIELD (µg)
Rat Tail	30-40	4-9
Rat Liver	15-20	20-38
Rat Heart	30-40	6 -14
Rat Lung	15-20	40-58

After Sample Preparation

6.1 Sample Addition

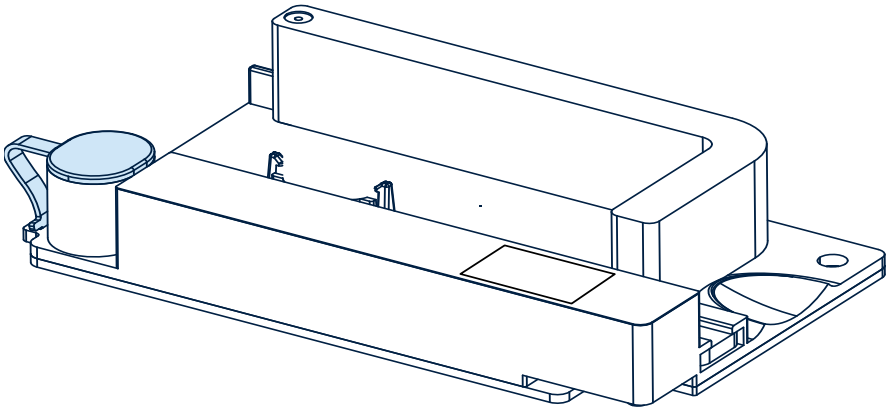
1. Open the sample well cap and transfer all of the prepared sample volume (as per section 5) into the sample well.

   Note. Ensure the pipette tip does not touch the rim of the sample well or the air hole within the sample well.



2. Once the sample has been added, close the sample well cap tightly by pushing the cap down for at least 1 second.

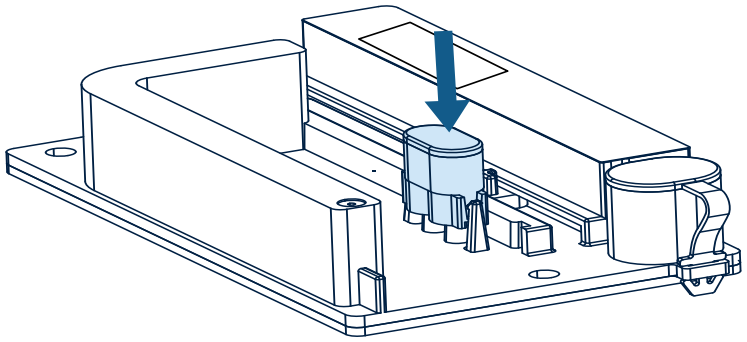
   Note. In case of a "Check sample cap"error message take out the X8 Cartridge and re-check to make sure the sample cap is sealed tightly before inserting the Cartridge into the bay.



6.2 LyoCap Assembly

1. Next open the LyoCap pouch and retrieve the LyoCap. Press the LyoCap (foil side down) onto the capsule holder on the X8 Cartridge.

   Note. There is no specific orientation to install the LyoCap. Minimize the time between LyoCap assembly and starting X8 run.



6.3 Starting the X8 Run

1. The X8 Cartridge is now ready to use with the X8 OneTouch Instrument.
2. Please place a sterile microcentrifuge tube in the tube holder before inserting the X8 Cartridge.
3. For the operation of X8 OneTouch Instrument, please refer to the X8 OneTouch Operating Manual (CGU-0001).
4. For the selection of protocols based on the sample types, please refer to the Appendix for the X8 Protocols.

6.4 End of the X8 Run

1. When the run is completed, the LED indicator will turn BLUE and a "Done" message will show on the X8 App.
2. Remove the X8 Cartridge from the bay.
3. Carefully remove the microcentrifuge tube from the tube holder and close the cap tightly.

   Note. If sample eluates are not processed immediately, store the eluted DNA at 4 °C.

6.5 Disposal of X8 Cartridge

1. Treat the used X8 Cartridge as a biohazard and discard as per local and organizational guidelines. Please refer to X8 Tissue DNA Cartridge Kit Safety Data Sheet (CGU-0009).

Appendix

7.1 X8 Protocols

SAMPLE TYPE	TOTAL WEIGHT/ CONC.	PROTOCOL NAME	ELUTION VOLUME
Rotor-stator homogenized tissue	10-30 mg	TD_MT_ Homegenizer_V#_#	150 - 200 µL
Heat block dissociated tissue	10-30 mg	TD_MT_ Heatblock_V#_#	150 - 200 µL
Gram Positive	1 x 10^9	TD_GP_V#_#	150 - 200 µL
Gram Positive	> 0.1 x 10^9 to < 1x 10^9	TD_GP_V#_#_Low_EB	50 - 100 µL

1. For bacterial cells concentration between 0.1 x 10^9 to less than 1x 10^9, please use Low EB protocol and Elution Buffer volume of 50 to 100 µL.
2. The "…V#_#"in the protocol name refers to the version of the protocol.

Troubleshooting

For additional technical information or advice, please contact our Customer Technical Support Division.

Email: support@onebiomed.com

TECHNICAL PROBLEM	DESCRIPTION
"Check sample cap" error	Reapply the sample cap firmly. Ensure that there is no visible air gap between the pierced foil and the tape.
Lower than expected DNA yield	
Incorrect sample amount Incorrect X8 protocol used	The genomic DNA yield from samples depends on the sample concentration and the corresponding X8 protocol. Refer to Appendix for the recommended sample concentration and X8 protocol. Excessive pretreatment may result in low DNA yield and quality. Please refer to section 5 for sample preparation.
DNA degradation after storage	It is recommended to store the eluted DNA at 4 °C.
Sample did not fully enter the channel	Maximum 250 µL of sample volume is to be added, ensure there are no air bubbles while loading into the sample well. Avoid touching the airhole while loading into the sample well. Avoid introduce undissociated tissue into the sample well.

Lower than expected purity ratios	
Sample overload	Refer to Appendix for the recommended sample amount and X8 protocol.
Compromised washing	Improper storage or handling of the X8 Cartridges can introduce reagent blockage.
Inefficient tissue lysis	For tougher tissue samples, a longer pretreatment may be needed with the lysis buffer and Proteinase K for proper cell lysis. 1. Cut tissue into smaller pieces to facilitate lysis. 2. Ensure that the sample is fully submerged in the Lysis Buffer containing Proteinase K. 3. After lysis, vortex the sample well; this will not damage or reduce the size of the DNA.
Viscous eluate	Ensure that no more than 10-40 mg of tissue or 1 x 10^9 bacterial cells are used per sample.
Elution Buffer related issues	
Too much or no elution	Improper storage or handling orientations may result in reagent blockage in the X8 Cartridge.
Too little or no elution	Incorrect addition of the elution buffer. Please refer to section 4.2 for Elution Buffer Addition. There will be dead volume retained in the X8 Cartridge channel after elution of nucleic acid.

RESOURCES

We are dedicated to helping you get the most out of your Xceler8 Platform by offering multiple helpful resources:

Products page

Explore our ever-growing portfolio of products that Xceler8 your sample preparation workflow.

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