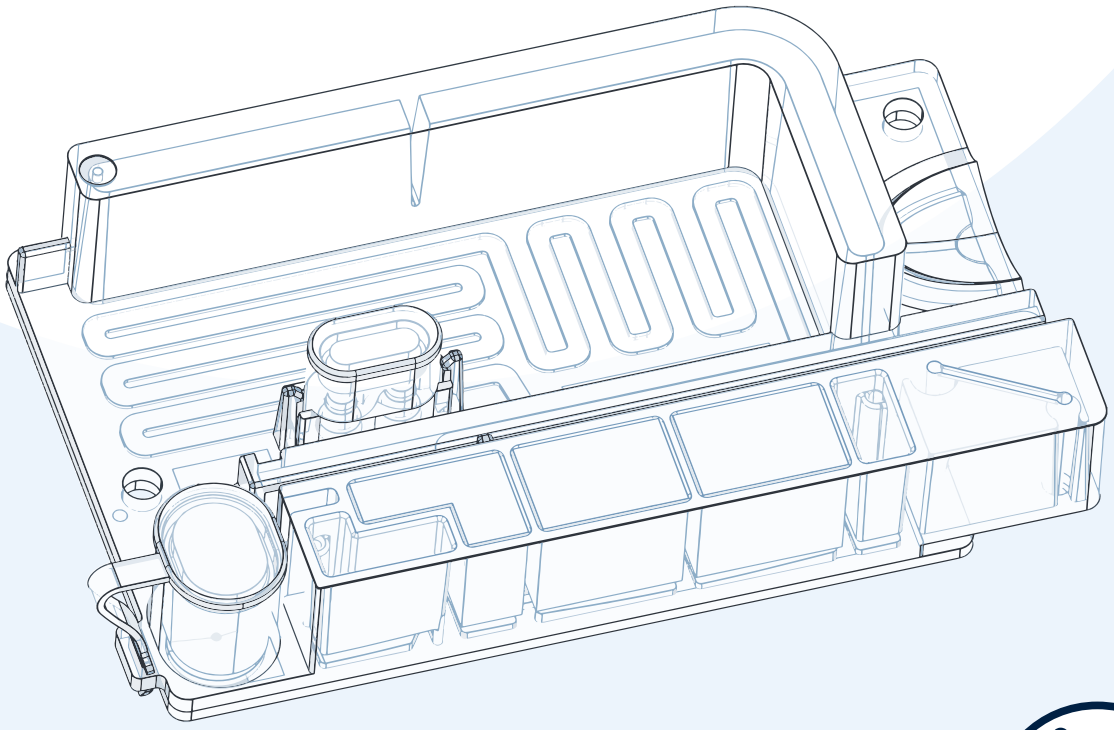
X8™ Cellular RNA Cartridge Kit

User Guide

FOR USE WITH:

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



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Manual Part Number

CGU-0007-EN *Rev B*

Legal Notices

Customer Information and Feedback

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

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Revision Summary

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Effective Date

August 31, 2023

Revision

- Complete Document Overhaul

Introduction

1.1 Cartridge Kit Overview

The X8™ Cellular RNA Cartridge Kit is used with the X8™ OneTouch Instrument to provide a simple method for efficient, automated extraction of cellular RNA from mammalian cell culture samples.

The X8 Cartridge Kits include all necessary reagents for high-quality total cellular RNA extraction. The lysis buffer has been optimized to minimize cellular DNA co-purification. 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 cellular RNA 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 cellular RNA extraction and purification. The novel chemistry-based approach purifies cellular RNA using a reversible cross-linker. It selectively binds & clusters the released cellular RNA from the lysed sample onto the cartridge's channel. Once impurities are washed away & sealed in the built-in waste reservoir, the purified cellular RNA is released and eluted using an elution buffer.

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1.2 Intended Use

The X8 Cellular RNA Cartridge Kit (Cat# X8-CR-001-24) is intended **For Research Use Only. Not for use in diagnostic procedures.** The X8 Cellular RNA Cartridge Kit has been designed for automated extraction of total cellular RNA from mammalian cell culture samples using the X8 OneTouch Instrument (Cat# X8-OT-101-IN).

1.3 Product Use Limitation

The X8 Cellular RNA Cartridge Kit (Cat# X8-CR-001-24) is not intended for use with sample types other than mammalian cell culture.

1.4 Storage of Eluted RNA

If sample eluates are not processed immediately, store the eluted RNA at -80 °C.

Components & Storage

2.1 Cartridge Kit Components

The X8 Cellular RNA Cartridge Kit contains 24 Cartridges that are designed for single-use only.

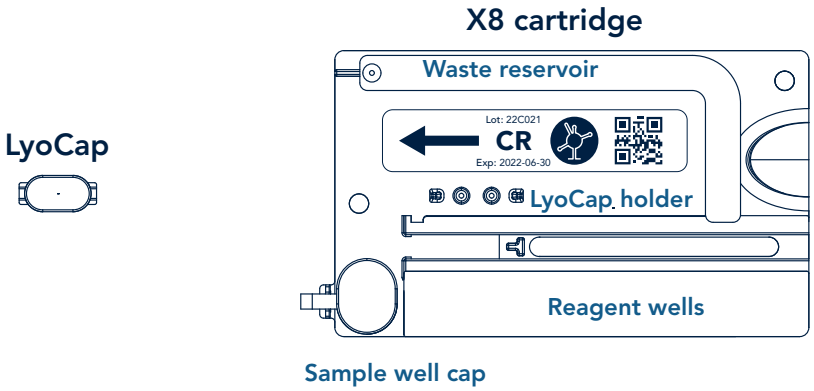
The X8 Cellular RNA Cartridge Kit includes:

- 24 - X8 Cellular RNA Cartridge Pouches (PN. X8-CR03001-24)
- 9 mL - C-RNA Lysis Buffer (PN. X8-LB2)
- 5 mL - RNA Elution Buffer (PN. X8-EB2)

2.2 Cartridge Pouch Components

The X8 Cellular RNA Cartridge Pouch includes:

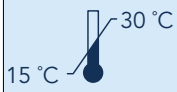
- 1 - X8 Cellular RNA Cartridge (PN. X8-CR03001)
- 1 - LyoCap pouch that contains a cross-linker bead



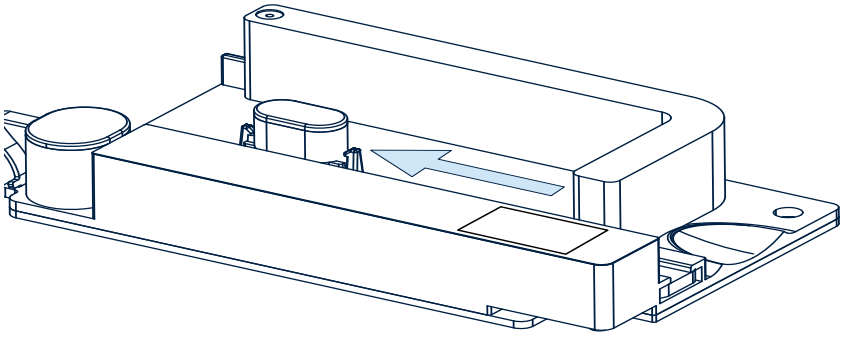
X8 Cartridge Pouch Components

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 Cellular RNA 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 local and institutional guidelines for the handling and disposal of all infectious substances.
	**Storage conditions.** The X8 Cellular RNA 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.

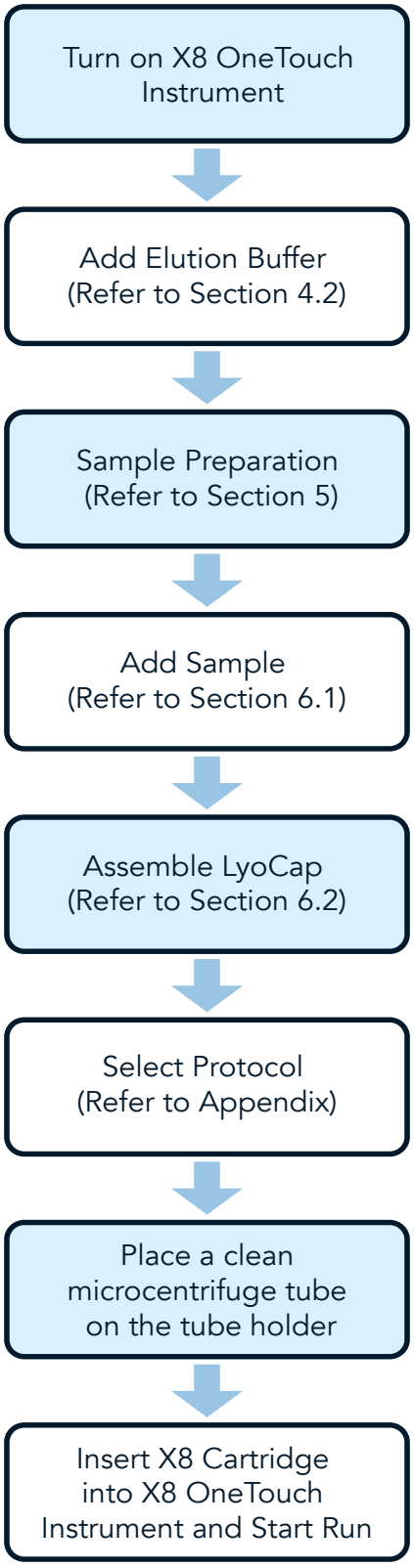


Optimal storage and handling orientation.
Set up on a flat surface with the label-side facing up.

For more information on safe handling, please refer to the Product Safety Data Sheets (SDS, CGU-0008) available at www.onebiomed.com/products.

X8 Workflow

3.1 Workflow



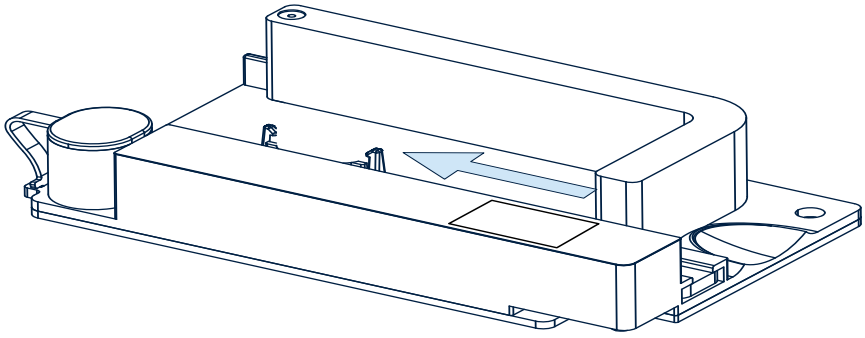
Before Each Run

4.1 Turn on X8 OneTouch

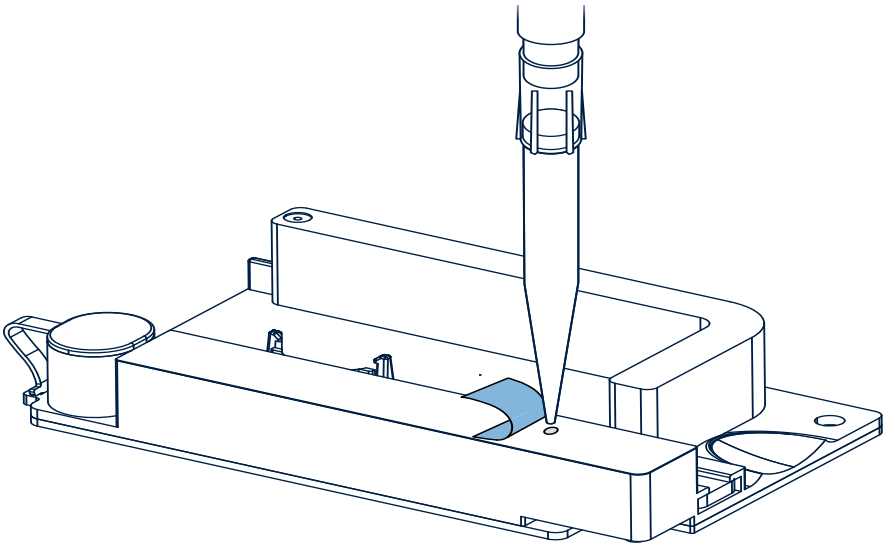
Ensure the X8 OneTouch Instrument is turned on and equilibrated. Please refer to the X8 OneTouch Operating Manual (CGU-0001) for additional details.

4.2 Elution Buffer Addition

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



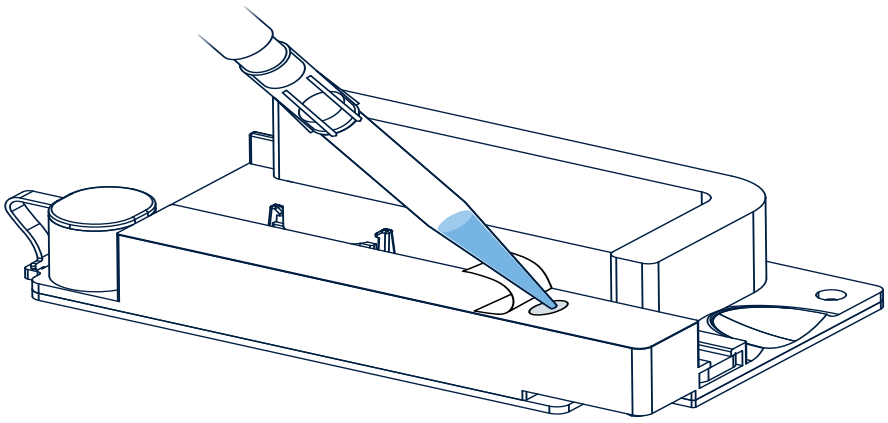
2. Gently lift the blue sticker tab on the X8 Cartridge and pierce the foil at the marked position using a sterile pipette tip.



3. Carefully aspirate 100 µL of the RNA Elution Buffer (PN. X8-EB2), 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 RNA Elution Buffer into the well without introducing any air bubbles.

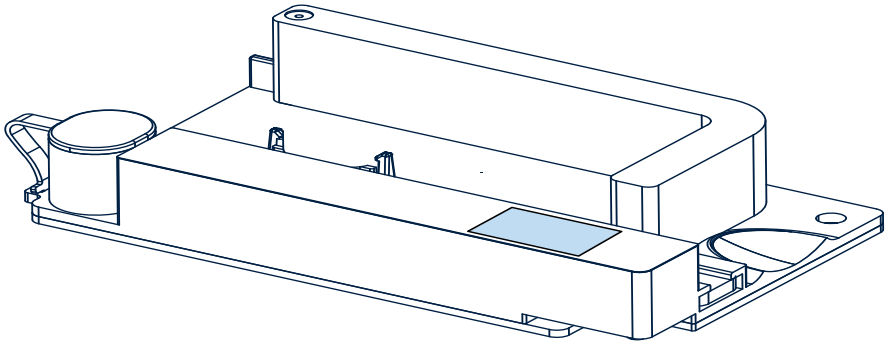


   Note. Incorrect addition of the Elution Buffer into the X8 Cartridge can result in little or no elution of the purified nucleic acid.

5. Peel the backing paper on the tape and apply the tape to seal the pierced foil. Run your finger across the tape 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 Materials

1. Micropipettes and sterile pipette tips.
2. Sterile microcentrifuge tubes (0.5 mL or 1.5 mL).
3. 1X PBS.
4. Centrifuge (with adapter for 1.5 mL / 2 mL microcentrifuge tubes).
5. Vortexer

5.2 Cell Culture

1. Obtain the cells of interest in suspension.
2. Centrifuge to pellet the cells and discard supernatant. Recommended speed: 200 x g for 5 minutes.
3. Wash the cells by adding at least 1 mL of PBS per 1 x 10^6 of cells and mix gently by pipetting to resuspend cells. Repeat step 2.
4. Determine the number of cells and resuspend the cell pellet to get 1 x 10^6 to 2 x 10^6 cells in 30 µL of 1X PBS. Add 280 µL of RNA Lysis Buffer and short pulse vortex 5 times to resuspend the cells.

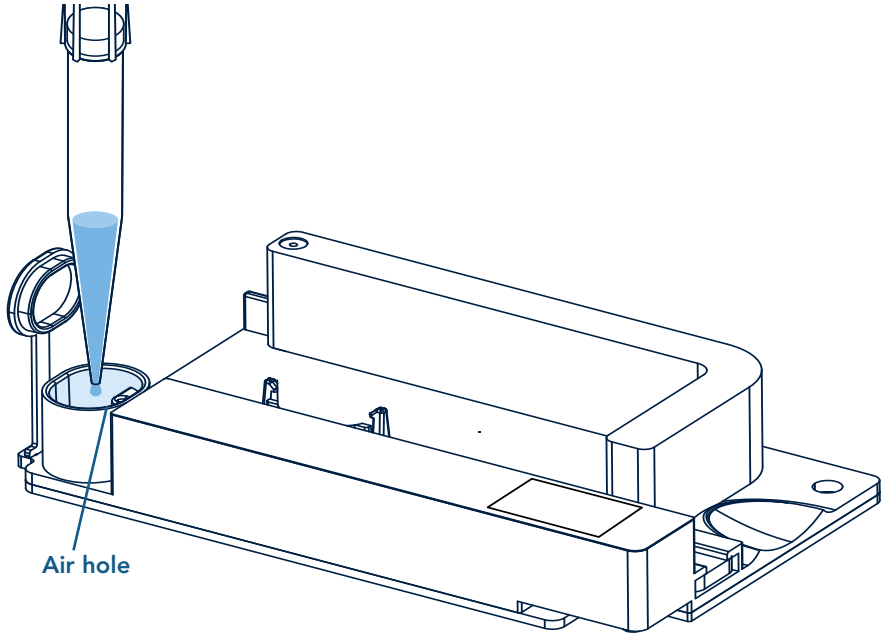
   Note. Proceed to section 6.1 immediately after RNA Lysis Buffer addition.

After Sample Preparation

6.1 Sample Addition

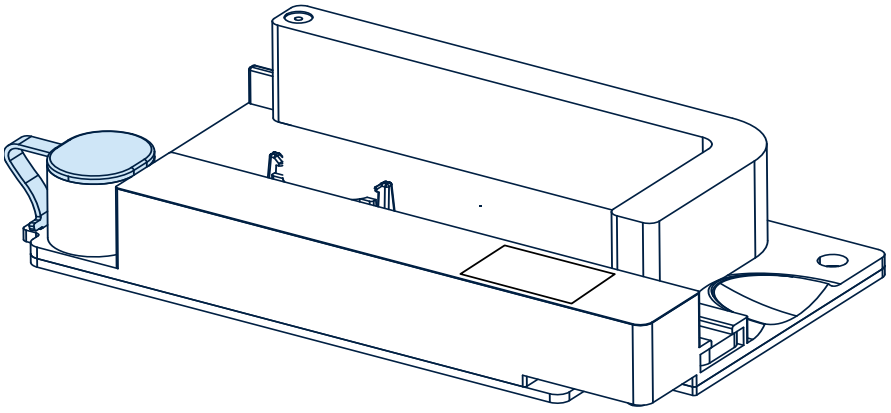
1. Open the sample well cap and transfer all of the prepared sample volume (as per section 5) into the sample well.

   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.

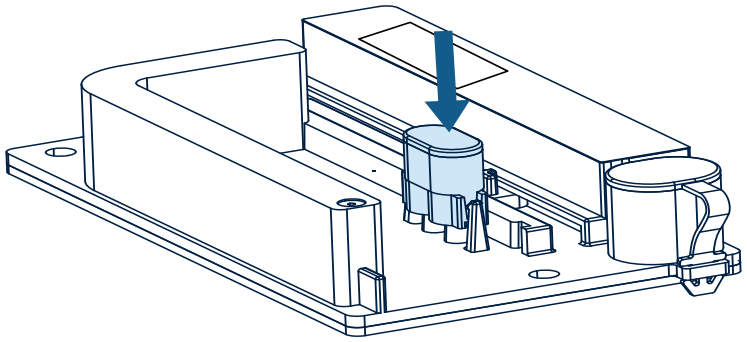
   Note. In case of a "Check sample cap"error message, take out the X8 Cartridge and re-check to make sure the sample cap is sealed tightly before inserting the Cartridge into the bay.



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 for 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 RNA at -80 °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 Cellular RNA Cartridge Kit Safety Data Sheet (CGU-0008).

Appendix

7.1 X8 Protocols

SAMPLE TYPE	TOTAL CELL CONC. INPUT	PROTOCOL NAME	ELUTION VOLUME
Cultured cells	1 x 10^6 - 2 x 10^6	CR_CC_V#_#	50 - 100 µL

1. 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 RNA yield	
Incorrect storage of samples	Storage of sample was not optimal. Use fresh cells whenever possible.
Incorrect sample concentration	The yield of RNA from cultured cell samples depends on the cell type and the number of cells processed. Increase cell numbers in the starting material up to 2 x 10^6 cells to increase yield of purified nucleic acid.
RNA degradation after storage	It is recommended to store the eluted RNA at -80 °C. Avoid multiple freeze-thaw of the samples

Sample did not fully enter the channel	Maximum 310 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.
More than the recommended amount of short pulse vortex	Shorten the duration of each pulse vortex and do not pulse vortex for more than 5 times
Lower than expected purity ratios	
Sample overload	Refer to Appendix for the recommended sample concentration and X8 protocol.
Compromised washing	Improper storage or handling of the X8 Cartridges may result in reagent blockage in the X8 Cartridge.
Viscous eluate	Ensure that no more than 2 x 10^6 cultured cells are used per sample.
Elution Buffer related issues	
Too much 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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