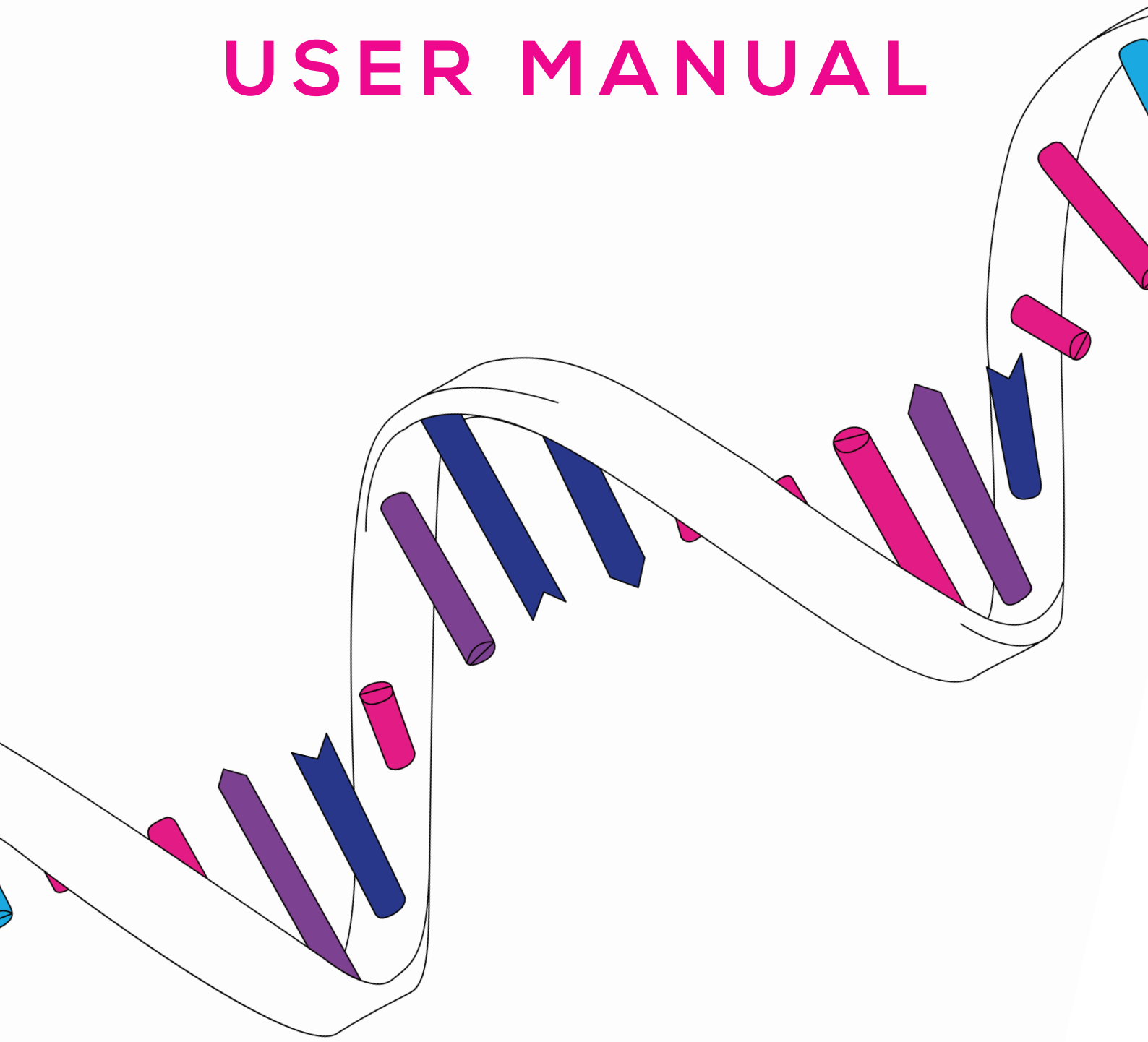
qEV RNA EXTRACTION KIT

USER MANUAL



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The qEV RNA Extraction Kit is produced by Norgen Biotek Corp. for Izon Science.

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1 / DEFINITIONS AND WRITING CONVENTIONS

Make sure to follow the precautionary statements presented in this guide. Safety and other special notices will appear in boxes and include the symbols detailed below.

Table 1: Safety and Hazard Symbols

	This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations
	This symbol indicates where special care should be taken

2 / INTRODUCTION

2.1 Intended Use

The qEV RNA Extraction Kit can be used for the extraction of RNA from EVs that have been isolated using qEV columns, and for EVs that have been concentrated after qEV isolation. The qEV RNA Extraction Kit enables the extraction of RNA from EVs isolated from biofluids as well as from cell culture media. The extraction provides samples of high purity which can be eluted into volumes of 50–100 µL (as specified by the user). The purified RNA is suitable for use in downstream applications including qPCR, microarrays, and RNA sequencing.

3 / MANUAL OPERATING INSTRUCTIONS

3.1 Working With RNA

RNases are very stable and robust enzymes that degrade RNA, and are not always sufficiently removed by autoclaving solutions and glassware.

Surface contamination. All surfaces should be cleaned with commercially available RNase decontamination solutions prior to starting experiments. RNA work should be conducted away from microbiological workstations.

Gloves. Always wear clean, disposable gloves, and change gloves frequently.

User provided equipment. RNase-/DNase-free solutions, tips and tubes, plus designated pipettes and lab coats should be used for all experiments involving RNA.

RNA stability. Purified RNA should be handled and kept on ice prior to and during downstream applications.

3.2 Kit Components

Components of the qEV RNA Extraction Kit (50 preparations)

Components	50 preparations
Lysis Buffer A	2 x 30 mL
Lysis Additive B	7 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
Mini Spin Columns	50
Collection Tubes	50
Elution Tubes (1.7 mL)	50

Lysis Buffer A contains guanidine thiocyanate and should be handled with care. Guanidine thiocyanate forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettes
- RNase-/DNase-free, sterile filter tips and pipettes
- Vortex
- 96%–100% Molecular Biology Grade Ethanol
- Sterile, RNase-/DNase-free tubes

4 / PROTOCOL

This protocol describes the method for RNA extraction from EVs purified using qEV columns.

Prior to RNA extraction:

- Store samples at -80°C.

- Prepare a working concentration of Wash Solution A by adding 90 mL of 96%–100% ethanol to the supplied bottle (containing 38 mL of concentrated Wash Solution A) to give a final volume of 128 mL.

Ensure that all solutions are at room temperature and that Lysis Buffer A is warmed to 60°C for 20 minutes prior to use. If precipitates are present, mix all solutions well until the solutions become clear.

- Thaw samples on ice prior to RNA extraction.

For successful RNA extraction, the following practices are recommended:

- Perform all centrifugation steps at room temperature using appropriate tubes. *n.b., Spin columns should not be used with vacuum apparatus.*

- If any solutions do not pass through the spin columns within the specified centrifugation time, spin for an additional 1–2 minutes until the solution completely passes through the column. Do NOT exceed the centrifugation speed.

After applying the Elution Solution A, allow to stand for 2 minutes prior to centrifugation. This is an essential step in maximising yield. Do not skip or shorten this step.

4.1 Procedure

If using the qEV Concentration Kit, separate the EV-bound Nanotrap® particles from the supernatant by centrifuging the particle-sample suspension at 16,800 x g for 10 minutes at room temperature. Without disturbing the Nanotrap® EV particle pellet, remove the supernatant (either transfer to a clean tube for analysis of non-EV components of the sample, or discard). The remaining Nanotrap® EV particle pellet is now ready for the RNA extraction procedure.

1. To your chosen volume of qEV isolate, add 1.5 volumes of Lysis Buffer A and 0.2 volumes of Lysis Additive B. This will be your **Lysed EV Solution**.

   E.g., if you have 200 µL of qEV isolate, add 300 µL of Lysis Buffer A and 40 µL of Lysis Additive B, for a total Lysed EV Solution volume of 540 µL.

n.b., When beginning with an EV pellet such as the Nanotrap® EV particle pellet, add 250 µL of Lysis Buffer A and 20 µL of Lysis Additive B directly to the EV pellet to produce your Lysed EV Solution.

2. Mix the Lysed EV Solution well by vortexing for 10 seconds, then incubate at room temperature for 20 minutes.

3. Following incubation, add 0.93 volumes (using the volume of the Lysed EV Solution as a reference volume) of 96%-100% ethanol to Lysed EV Solution and mix well by vortexing for 10 seconds. This is your Lysed EV Ethanol Solution.

   E.g., to 540 µL of Lysed EV solution, add 502.2 µL of ethanol for a final volume of 1042.2 µL.

4. Transfer up to 700 µL of the Lysed EV Ethanol Solution into the mini spin column. Centrifuge for 1 minute at 3,300 x g. Discard flowthrough.

5. Repeat step 4 until all Lysed EV Ethanol Solution has been run through the mini spin column.

6. Apply 600 µL of Wash Solution A to the column and centrifuge for 30 seconds at 3,300 x g, then discard flowthrough.

7. Repeat step 6.

8. Spin the empty column for 1 minute at 13,000 x g to remove any remaining liquid. Discard collection tube and transfer the spin column into a fresh 1.7 mL elution tube.

9. Apply a chosen volume of Elution Solution A between 50 and 100 µL to the column and centrifuge for 1 minute at 400 x g followed by a further 2 minutes at 5,800 x g.

A second elution can be performed if deemed necessary to further maximise recovery. For this, take the eluate from step 9 (i.e., the same Elution Solution A that has already passed through the column) and re-apply it to the column. Incubate the column for 2 minutes at room temperature and centrifuge to re-collect as in step 9. Do not use new Elution Solution A for this optional step if you wish to increase concentration as this will result in a dilution.

Following extraction, RNA should be stored at -80°C until it is required for downstream applications.

5 / FREQUENTLY ASKED QUESTIONS

What happens if I use the wrong centrifuge speed?

Centrifuging the mini spin columns at a speed higher than recommended may affect RNA yield. Centrifuging at a speed lower than recommended will not affect RNA yield but may increase the elution time.

What happens if I perform the centrifugation at the wrong temperature?

We recommend performing all centrifugation steps at room temperature; however, centrifugation at 4 °C will not adversely affect the kit performance.

How does Elution Solution A volume impact upon RNA?

Eluting your RNA in high volumes will increase the yield but the resulting sample will have a low concentration of RNA. Eluting in small volumes will increase the concentration but will reduce the overall yield.

What happens if I forget to do a dry spin before my final elution step?

Purified RNA will be contaminated with the Wash Solution A which may reduce the quality of the purified RNA and interfere with downstream applications.

Why do my samples show a low RNA yield?

The amount of RNA contained within EVs is very small and can vary depending on the sample that they were isolated from and purification methods used. The yield of RNA can be increased by increasing the amount of sample input. For optimal and standardised results, the qEV RNA Extraction Kit should be used in combination with **qEV columns** and the **qEV Concentration Kit**.

Why is the A260/280 ratio of the purified RNA not 2.0?

Measurement of A260/A280 ratios are inaccurate at low RNA concentrations, like the ones you are likely to see from EV-RNA isolation. This is reflected in a low A260/A280 value and is acceptable for downstream analysis. If the A260/A280 value is high, this indicates contamination. In this case, run the eluted RNA through the extraction procedure again from Step 3 onwards. This may reduce yield but should increase purity.

What happens if I use a different elution buffer?

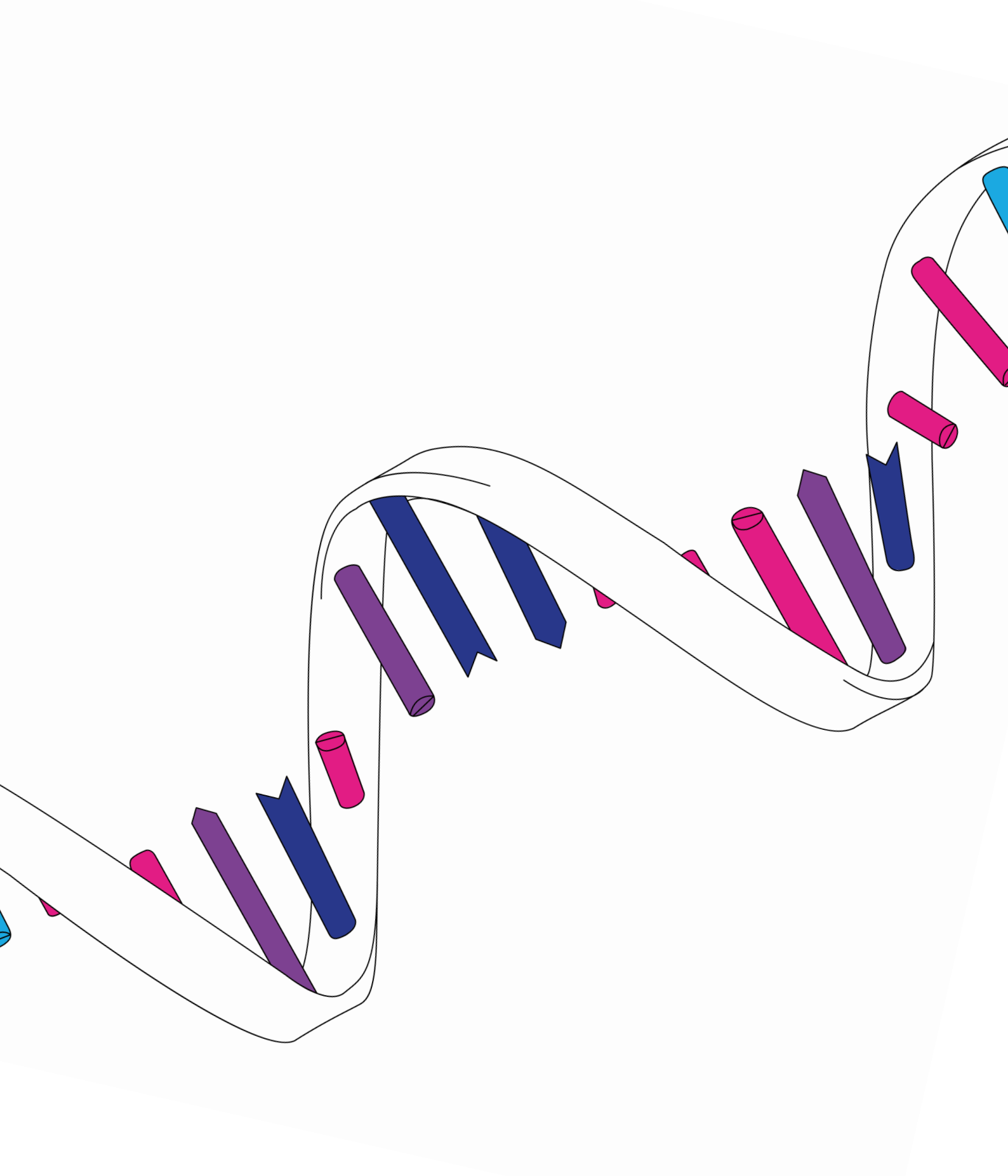
If an elution buffer other than the one provided in the kit was used, it may interfere with downstream applications. Common components that are known to interfere are high concentrations of salt (including EDTA), detergents and other denaturing agents. Check the compatibility of your elution buffer with the intended use.

6 / OTHER RESOURCES

Refer to the Safety Data Sheet for classification and labelling of hazards and precautionary statements.

The Safety Data Sheet for the qEV RNA Extraction Kit is located at support.izon.com/safety-data-sheets

For more information or to contact a customer support representative please visit support.izon.com



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