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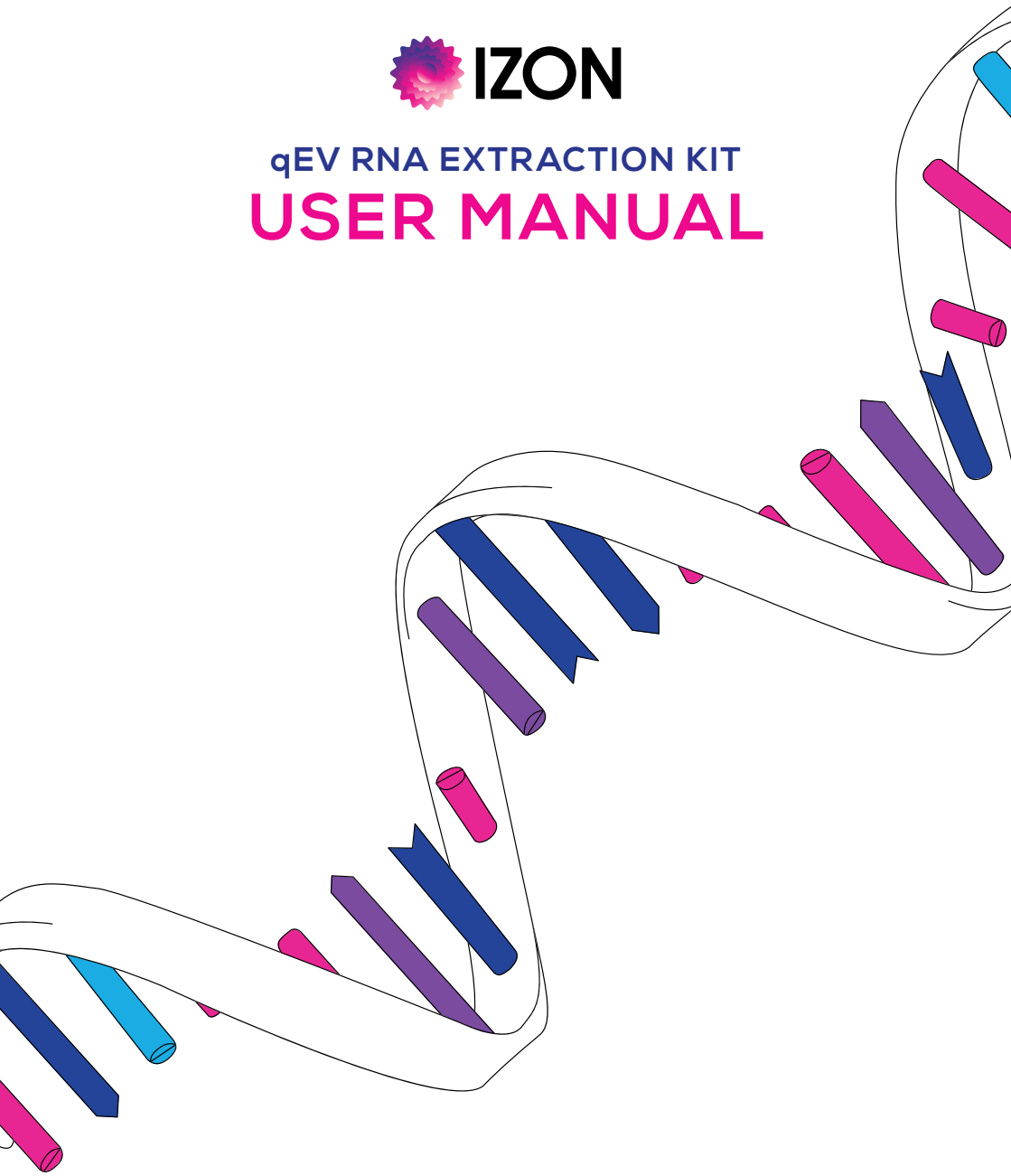
qEV RNA EXTRACTION KIT

USER MANUAL



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SPECIFICATIONS AND OPERATIONAL GUIDE FOR qEV RNA EXTRACTION KIT

RAPID & STANDARDISED EXTRACTION OF EXTRACELLULAR VESICLE RNA

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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.

Table 2: Terminology Used in this Manual

TERM	DEFINITION
RNA	Ribonucleic acid
EV	Extracellular vesicles
qEV Isolation column	Izon's size exclusion columns which isolate EVs from various fluids as they pass through a column packed with porous, polysaccharide resin

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 Isolation columns and concentrated (if necessary). The qEV RNA Extraction Kit enables extraction of RNA from EVs isolated from biofluids as well as from cell culture media. The extraction is highly specific, not compromised by size bias and 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 (RNA-seq) for purposes including monitoring of disease progression and therapeutic responses.

2.2 Quality Control

In accordance with Izon's ISO 13485-certified Quality Management System, each batch of the qEV RNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

2.3 Product Use Limitations

The qEV RNA Extraction Kit is designed for research purposes only. It is not intended for human or diagnostic use.

3 / OPERATING INSTRUCTIONS

The following section provides instructions for the use of qEV RNA Extraction Kit.

3.1 Working with RNA

RNases are very stable and robust enzymes that degrade RNA which are not always sufficiently removed by autoclaving solutions and glassware. Before attempting to extract RNA from EV samples, an RNase-free environment must be created. The following precautions are recommended as your best defence against these enzymes.

- The RNA area should be located away from microbiological workstations.
- Clean, disposable gloves should always be worn. Gloves should be changed frequently to avoid contamination.
- Designated solutions, tips, tubes, lab coats, pipettes, etc. should be used for all experiments involving RNA. Plasticware provided by user (e.g. 1.5, 2.0, 15, 50 mL tubes or pipette tips) should be certified RNase/DNase-free.
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade RNase/DNase-free water.
- All surfaces should be cleaned with commercially available RNase decontamination solutions prior to starting experiments.
- 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 preps)

Component	Quantity (50 preps)
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 pieces
Collection Tubes	50 pieces
Elution tubes (1.7 mL)	50 pieces

Lysis Buffer A contains guanidine thiocyanate and should be handled with care. Guanidine thiocyanate forms highly reactive, toxic 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
- Sterile filter tips and pipette
- Vortex
- 96%–100% Molecular Biology Grade Ethanol

4 / PROTOCOL

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

Prior to use, please ensure the following:

- EV samples have been stored at -80 °C prior to use (failure to do so will affect RNA yield).
- 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.
- Prepare a working concentration of the Wash Solution A by adding 90 mL of 96%–100% ethanol to the supplied bottle (which contains 38 mL of concentrated Wash Solution A) to give a final volume of 128 mL.
- All centrifugation steps are performed at room temperature in appropriate tubes.
- The provided spin columns are optimised for use with benchtop centrifuges and cannot be used with vacuum apparatus.
- 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.
- 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.

Procedure:

1. Add 900 µL of Lysis Buffer A and 125 µL of Lysis Additive B to 600 µL EV sample isolated using qEV Isolation columns and concentrated if necessary.
2. Mix the EV and Lysis Buffer/Additive solution well by vortexing for 10 seconds then incubate at room temperature for 20 minutes.
   Note: If using the qEV Concentration Kit, separate the depleted Nanotrap® particles from your extracted sample by centrifuging the particle-sample suspension at 16,800 x g for 10 minutes at room temperature. Without disturbing the Nanotrap® EV particle pellet, transfer the extracted sample supernatant into a clean tube.
3. Add 1.5 mL of 96%–100% ethanol to the mixture from Step 2 and mix well by vortexing for 10 seconds.
4. Transfer 700 µL of the mixture from Step 3 into a mini spin column. Centrifuge in a bench-top centrifuge for 1 minute at 3,300 × g. Discard the flowthrough and reassemble the mini spin column with its collection tube.
5. Repeat Step 4 as many times as necessary to transfer the remaining mixture from Step 3 into the mini spin column and discard the flowthrough.
6. Apply 600 µL of Wash Solution A to the column and centrifuge in a bench-top centrifuge for 30 seconds at 3,300 × g. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat Step 6 once more and discard the flowthrough.
8. Spin the empty column for 1 minute at 13,000 × g to remove any remaining liquid. Discard the collection tube.
9. Transfer the spin column to a fresh 1.7 mL elution tube. Apply 50 µL of Elution Solution A to the column and centrifuge for 1 minute at 400 × g, followed by a further 2 minutes at 5,800 × g.
10. For maximum recovery, transfer the eluted solution back to the column and let the column stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 × g, followed by a further 2 minutes at 5,800 × g.
11. The extracted RNA is now ready for downstream applications.

5 / FREQUENTLY ASKED QUESTIONS (FAQS)

What happens if a variable speed centrifuge is not available?

A fixed speed centrifuge can be used as long as the speed does not exclude our recommendations; however, reduced yields may be observed.

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.

What happens if I add more or less of the specified reagents?

This may reduce both the quality and quantity of the purified 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.

Can I perform a second elution?

Yes, but it is recommended that the second elution is in a smaller volume (e.g. 50% of first elution). It is also recommended to perform the second elution into a separate elution tube to avoid diluting the first elution.

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. The yield of RNA can be increased by increasing the amount of sample input; however, for optimal and standardised results the qEV RNA Extraction Kit should be used combination with the qEV Isolation columns and qEV Concentration Kit.

Why is the A260/280 ratio of the purified RNA lower than 2.0?

Most RNA extracted from EVs will be short RNA fragments present in very low concentrations. The A260/280 ratio tends to decrease with decreased RNA concentration; it is normal to find an A260/280 ration of 1–1.6 for your purified sample. This low A260/280 ratio will not affect any downstream application.

Why does my extracted RNA not perform well in downstream applications?

If an elution buffer other than the one provided in the kit was used, it may interfere with the downstream application. 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 www.izon.com/products/sds

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