Heidelberg Biolabs

LABORATORY MANUAL

BioLiqX RNA Isolation Kit

(for purification of total RNA from liquid samples and biological fluids)

Catalog Number: HB205A

Table of Contents

1. OVERVIEW

BioLiqX RNA Isolation Kit includes all the components required to isolate total RNA from liquid samples including blood plasma and serum. The method is based on prior lysis of a sample with phenol-containing **BioliqX Lysis Buffer R**, subsequent phase separation and spin-column purification of RNA. The protocol efficiently denatures protein-rich biological fluids and ensures the complete inactivation of RNases. Furthermore, the optimized composition of the **BioliqX Lysis Buffer R** enables superior recovery of highly diluted ultra-short RNA fragments and almost complete removal of the DNA during organic extraction. In addition, optimized washing buffers ensure the absence of enzyme-inhibiting traces of phenol in the RNA eluates. Finally, ultra-clean spin columns secure the absence of contaminating RNA and allow sample elution into the minimal volume of as little as 10 µL. The whole procedure can be completed within approximately 1.5 hours and requires a hands-on time between 10-30 minutes depending on the number of samples.

The kit is delivered in a compact carton box containing 50x **Spin Columns**, 50x **Collection Tubes**, 50x **Nuclease-Free Tubes**, **RNAse-Free Water** as well as ethanol-free concentrates of **Washing Buffer 1** and **Washing Buffer 2**. The phenol containing **BioliqX Lysis Buffer R** is supplied in a separate dark glass bottle.

The following components are **not** included and have to be supplied by the user:

- Safe-Lock 2 mL microcentrifuge tubes for the initial sample lysis.
- Molecular biology grade absolute ethanol.
- Molecular biology grade chloroform.

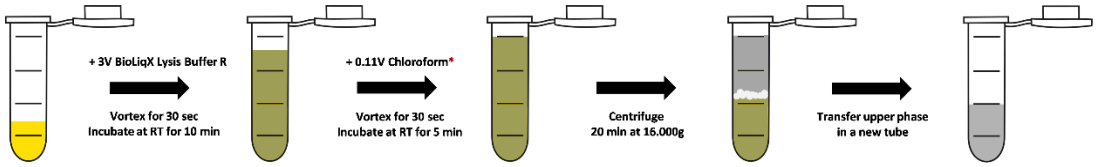
The volume of the initial sample can be custom but will be limited by the volume of the microcentrifuge tube used for the initial sample lysis. While the kit is designed for 400 µL sample volume in a 2.0 mL microcentrifuge tube, various other inputs can be used (e.g. 200 µL of sample in a 1.5 mL microcentrifuge tube). Importantly, the final sample volume should be at least 200 µL to minimize the loss of the aqueous upper phase after organic extraction. We highly recommend using a synthetic spike-in cel-miR-39 (or similar small ssRNA) control for (1) assessing the efficacy of the RNA isolation between samples, and (2) normalization of miRNAs (or other ssRNAs) expression in biological fluids after downstream RT-qPCR. The synthetic spike-in cel-miR-39 control is included in BioLiqX cf-RNA Isolation QC Kit (cat # HB202A) which is highly recommended for assessing the isolation efficacy of cell-free RNA.

The procedure is suitable for use with biological fluids and other liquid samples containing citrate or EDTA. Plasma samples prepared from blood collected on heparin should not be used as heparin significantly interferes with multiple downstream applications including RNA sequencing and RT-qPCR.

2. THE METHOD WORKFLOW

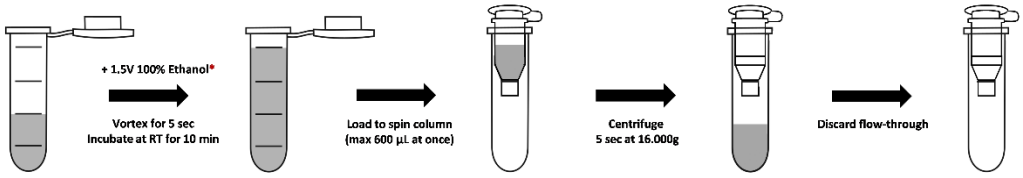
The procedure for RNA isolation using BioLiqX RNA Isolation Kit includes three general steps:

(i) Lysis and organic extraction



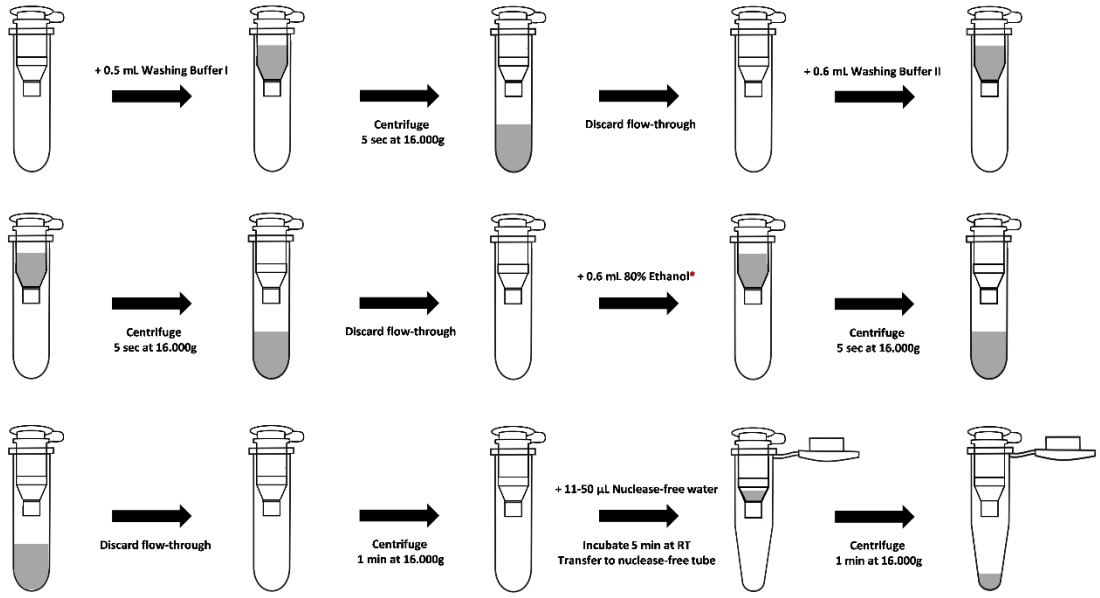
On the first step **(i)**, the sample is lysed in 3 volumes of **BioLiqX Lysis Buffer R** and thoroughly mixed. The lysate is then separated into upper aqueous and lower organic phases by adding 0.11 volumes of **chloroform** and high-speed centrifugation. The solid interphase containing some of the proteins and the DNA can be also formed after centrifugation in some samples. The upper phase containing the RNA is collected for the downstream procedure while the lower phase and the interphase are discarded.

(ii) Binding to spin column



In the second step **(ii)**, the 1.5 volumes of 100% **ethanol** are added to the upper phase to facilitate the binding of RNA molecules to the silica support. The sample is then applied to a corresponding spin column, where the RNA binds to the membrane while other contaminants including the traces of phenol remain in the flow-through.

(iii) Spin column washing and elution



On the final step **(iii)**, the spin column is washed sequentially with **Washing Buffer I**, **Washing Buffer II** and 80% **ethanol**, and the high-quality RNA is eluted in the appropriate volume of **RNase-Free Water**.

Phenol-free RNA isolation from protein-rich liquid samples such as blood serum and plasma can be associated with dramatic losses of RNA during the protein precipitation step. Phenol-chloroform extraction does not imply the removal of proteins by precipitation and, thus, remains superior to phenol-free protocols in terms of RNA recovery.

3. KIT COMPONENTS AND STORAGE

Component	Catalog No	Size/Volume
BioLiqX Lysis Buffer R	HBLBR20A	65 mL
Spin Columns	HBSC20A	50 items
Washing Buffer I	HBWB120A	12 mL
Washing Buffer II	HBWB220A	40 mL
Collection Tubes	HBCT20A	50 items
RNase-Free Water	HBWT20A	10 mL
Nuclease-Free Tubes	HBTU20A	50 items

All components of the BioLiqX RNA Isolation Kit should be stored at room temperature.

4. REQUIRED MATERIALS NOT INCLUDED

Check to ensure that all necessary materials and equipment are available before starting with RNA isolation. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

General laboratory equipment and consumables

- Proper fume-hood for working with organic solvents including phenol.
- Microcentrifuge (12,000 x g - 16,000 x g, compatible with 1.5 mL and 2.0 mL microtubes).
- Single-channel pipettes for handling 100 µL to 1,000 µL volumes.
- Ribonuclease-free pipette tips (pipette tips with aerosol barriers highly recommended).
- Safe-lock 2.0 mL microcentrifuge tubes (recommended: Eppendorf cat # 0030123344).
- Vortex mixer.

Additional reagents

- Molecular biology grade absolute ethanol.
- Molecular biology grade 80% ethanol.
- Molecular biology grade chloroform.
- [OPTIONAL]. Appropriate spike-in small ssRNA control.

Cel-miR-39 ssRNA control can be purchased together with BioLiqX cf-RNA Isolation QC Kit (cat # HB213A), BioLiqX HS miRNA Assays (cat # HB214A) or BioLiqX Small RNA-seq Kit (cat # HB202A).

5. GENERAL GUIDLINES

We strongly recommend that you read the following warnings and precautions. If you need further assistance, you may contact us at info@heidelbergbiolabs.com

- Wear gloves at all steps to protect samples from contamination and degradation by nucleases
- Wear a protective mask which prevents breathing into the test tubes
- All containers and storage areas must be free of contaminants and nucleases
- All steps should be performed at room temperature (preferably 20°–25°C)
- Please read the complete manual before first time use

The volume of the initial sample can be custom but will be limited by the volume of the microcentrifuge tube used for the initial sample lysis. While the kit is designed for 400 µL sample volume in a 2.0 mL microcentrifuge tube, various other inputs can be used (e.g. 200 µL of sample in a 1.5 mL microcentrifuge tube). Importantly, the final sample volume should be at least 200 µL to minimize the loss of the aqueous upper phase after organic extraction. We highly recommend using a synthetic spike-in cel-miR-39 (or similar small ssRNA) control for (1) assessing the efficacy of the RNA isolation between samples, and (2) normalization of miRNAs (or other ssRNAs) expression in biological fluids after downstream RT-qPCR.

6. SAFETY GUIDLINES

To minimize hazards, ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Always wear a lab coat, disposable gloves and protective goggles when working with chemicals containing phenol and guanidine salts.
- Minimize the inhalation of chemicals.
- Do not leave chemical containers open.
- Work only under adequate ventilation conditions (fume hood).
- Handle chemical wastes in a fume hood.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- Biological fluids from human and animal subjects are considered potentially infectious. Ensure that all necessary precautions recommended by the appropriate authorities in the country of use are taken when working with biological fluids

For more information, please refer to the material safety data sheet available online at:

www.heidelbergbiolabs.de/onewebmedia/BioLiqX_RNA_Isolation_Kit_MSDS_031021.pdf

BioLiqX Lysis Buffer R and **Washing Buffer I** include guanidine isothiocyanate and are not compatible with disinfecting reagents containing bleach. If spilled, clean the affected surface first with suitable laboratory detergent and water, and then with 1% sodium hypochlorite to neutralize potentially infectious agents.

7. BEFORE START

- Please read the complete manual before first time use.
- Washing Buffer I and Washing Buffer II are supplied as concentrates. Before using for the first time add 10 mL 100% Ethanol to the 40 ml Washing Buffer I concentrate, and 48 mL 100% Ethanol to the 12 mL Washing Buffer II concentrate.
- Prepare 80% Ethanol using RNase-free water and molecular biology grade absolute (100%) ethanol.
- [OPTIONAL] Dilute cel-miR-39 spike-in control ssRNA in the appropriate aliquot of **BioliqX Lysis Buffer R** to the final concentration 1 fg/mL - 1 pg/mL.

8. PROTOCOL

1. Aliquot 400 µL of liquid sample into PCR clean 2.0 mL safe-lock microcentrifuge tube (not supplied).
2. Add 1200 µL (3 volumes) **BioliqX Lysis Buffer R** containing appropriate amount of diluted spike-in control ssRNA.
3. Mix by vortexing (or shake vigorously) for 30 sec and incubate at room temperature for 10 min.
4. Add 180 µL (approximately 0.11 volumes) **chloroform** (not supplied).
5. Mix by vortexing (or shake vigorously) for 30 sec and incubate at room temperature for 5 min.
6. Centrifuge the microtube(s) for 20 min sec at 12.000-16.000g at room temperature.
7. Transfer 400 µL of the upper aqueous phase to a new PCR clean 2.0 mL microcentrifuge tube (not supplied). Avoid transfer of any interphase material.
8. Add 600 µL (1.5 volumes) of **100% ethanol** (not supplied).
9. Mix by vortexing (or shake vigorously) for 30 sec and incubate at room temperature for 5 min.
10. Insert new spin column into the new collection tube.
11. Load up to 600 µl of the sample into the spin column and close the lid.
12. Centrifuge spin column(s) for 10 sec at 12.000-16.000g and discard the flow-through.
13. Repeat step 12 using the remainder of the sample and discard the final flow-through.
14. Load 500 µL **Washing Buffer I** to the spin column(s) and close the lid.
15. Centrifuge spin column(s) for 10 sec at 12.000-16.000g and discard the flow-through.
16. Load 600 µL **Washing Buffer II** to the spin column(s) and close the lid.
17. Centrifuge spin column(s) for 10 sec at 12.000-16.000g and discard the flow-through.
18. Load 600 µL **80% ethanol** (not supplied) to the spin column(s), close the lid and incubate at room temperature for 3 min.
19. Centrifuge spin column(s) for 10 sec at 12.000-16.000g and discard the flow-through.
20. Centrifuge spin column(s) additionally for 2 min at 12.000-16.000g to dry the membrane.
21. Discard the collection tube and place the spin column in a new nuclease-free tube.
22. Add 10 µL – 50 µL **RNase-Free Water** directly to the center of the spin column membrane.
23. Close the lid and incubate for 3 min at room temperature.
24. Centrifuge for 1 min at 12.000-16.000g to elute the RNA.

9. RNA STORAGE AND QUALITY CONTROL GUIDLINES

- Purified RNA can be stored in RNase-free water at temperatures from -20°C to -80°C for at least 1 year without detectable degradation.
- The total RNA yield may significantly vary between samples from different individuals as well as types of biological fluids.
- Total RNA content in cell- and cell-debris free blood plasma and serum are usually too low for quantification by optic density OD measurement devices (such as Nanodrop), fluorescent dyes measurement devices (such as Qubit) or capillary electrophoresis systems (such as Bioanalyzer and TapeStation).
- Purified plasma or serum RNA contains traces of proteins which will interfere with OD-based methods (such as Nanodrop), leading to significant overestimation of the purified RNA concentration.
- We recommend using synthetic spike-in ssRNA controls (for instance, cel-miR-39) and corresponding RT-qPCR-based measurement (e.g. BioLiqX cf-RNA Isolation QC Kit) to monitor efficacy of RNA isolation from biological fluids and other samples with low RNA content.

10. CONTACT INFORMATION

Heidelberg Biolabs GmbH
Im Neuenheimer Feld 582
69120 Heidelberg, Germany
Tel: +49 171 1908276
Fax: +49 6221 5025711
E-mails: info@heidelbergbiolabs.com, info@heidelbergbiolabs.de

NOTICE TO THE PURCHASER: LIMITED LICENSE FOR RESEARCH USE ONLY

The BioLiqX RNA Isolation Kit is intended for research and development purposes only, and is not suited for diagnostic applications. The purchase of this product conveys to the purchaser a limited, non-transferable right to perform internal research for the sole benefit of the purchaser. No right to resell or distribute this product or any of its components is conveyed expressly, by implication, or by estoppel. For information on purchasing additional rights, please contact Heidelberg Biolabs GmbH.

WARRANTY

The product needs to be handled by qualified personnel to ensure safety and proper use. Heidelberg Biolabs GmbH warrants that the product performs to the standards described in this guide until the expiration date, but does not provide any warranty if product components are misused, handled or stored improperly or replaced with substitutes. Under no circumstances shall the liability of this warranty exceed the purchase price of this product. We reserve the right to alter or modify products and services at any time to incorporate the latest technological developments without prior notice. The information in this user guide is subject to change without notice.

TRADEMARKS

LAST TIME REVISED: 23.01.2022