Genomic DNA removal and total RNA purification from all types of lysate (ISOLATE II Biofluids RNA Kit)

Bioline

Abstract

The ISOLATE II Biofluids RNA Kit is specially developed for the rapid phenol-free isolation of high quality total RNA from biofluids and viruses.

The following protocol is two part. The first part provides a procedure for the removal of genomic DNA applies to all the different types of lysate. Ensure you use the correct column: the Genomic DNA Removal Column has a **blue** ring.

The second part is the protocol for the purification of total RNA apply to all the different types of lysate. Ensure you use the correct column: the ISOLATE II RNA Column has a **black** ring.

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Guidelines

KIT CONTENTS

COMPONENT	50 Preps
ISOLATE II RNA Columns (black)	50
ISOLATE II Genomic DNA Removal Columns (blue)	50
Collection Tubes (2 mL)	100
Elution Tubes (1.7 mL)	50
Lysis Buffer RX*	40 mL
Wash Buffer W1† (concentrate)	38 mL
DNase I Solution (RNase-free)	0.8 mL
DNase I Reaction Buffer DRB	6 mL
RNA Elution Buffer	6 mL
Product Manual	1

*Contains a guanidine salt. Not compatible with disinfectants containing bleach or acidic solutions.

†Before use, add 90 mL of 96-100% ethanol and mark wash buffer bottle label.

DESCRIPTION

The ISOLATE II Biofluids RNA Kit allows convenient processing of multiple samples in 20 minutes without the use of laborious methods such as CsCl ultracentrifugation or handling of toxic chemicals such as phenol/chloroform.

The ISOLATE II Biofluids RNA Kit is specially developed for the rapid phenol-free isolation of high quality total RNA from biofluids and viruses. Total RNA can be purified from blood, plasma, serum and other types of biofluids such as saliva, urine, semen and cerebrospinal fluid (CSF). Viral RNA can be isolated from the same sample sources, as well as cultured cells and tissue. The kit isolates all sizes of RNA from large mRNA, viral RNA and ribosomal RNA (rRNA) down to small RNAs such as microRNA (miRNA) and short interfering RNA (siRNA).

Isolation is based on a fast spin column format using a novel RNA affinity resin as the separation matrix so that the small RNAs are preferentially purified from other cellular components. This kit does not require the use of phenol or chloroform.

Samples are first lysed in the presence of guanidinium thiocyanate, a chaotropic salt which immediately deactivates endogenous RNases to ensure purification of intact RNA. DNA is then captured on a Genomic DNA Removal Column to remove contaminating genomic DNA. Ethanol is added to the flow-through and then applied to an RNA Column. The bound total RNA is washed to remove any impurities and eluted. The RNA is of the highest quality and integrity and is ready for use in various applications, including:

- Real-Time PCR (qPCR)
- Reverse transcriptase PCR (RT-PCR)
- cDNA synthesis
- Next generation sequencing

- Northern blotting

- Microarray analysis

Please read this manual carefully to familiarize yourself with the ISOLATE II Biofluids RNA protocol before starting (also available on www.bioline.com). More experienced users can refer to the Bench-Top Protocol for quick referencing during the procedure.

STORAGE

Store DNase I at -20°C upon arrival. All other components should be stored at room temperature (18-25°C). Storage at lower temperatures may cause precipitation of salts.

SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Lysis Buffer RX contains guanidinium thiocyanate. This chemical is harmful in liquid form when in contact with skin or ingested. If the solution is allowed to dry, the powder is harmful if inhaled.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidinium salts. Reactive compounds and toxic gases can form. In the case of spillage, clean the affected area with a suitable laboratory detergent and water.

For detailed information, please consult the material data safety sheet (MSDS) available on our website at www.bioline.com.

PRODUCT SPECIFICATIONS

The ISOLATE II Biofluids RNA Kit is specially designed for the rapid isolation of total RNA including small RNAs (<200 nt). The kit is compatible with biofluids such as blood, plasma, serum, saliva, urine, semen and CSF. Viral RNA can also be extracted from the same sample sources. The preparation time is approximately 20 minutes for 10 preps. The isolated RNA is of high-purity (A_{260}/A_{280} ratio: >1.9) and high integrity (RIN>9) for high quality samples (see below) and is ready for use in various

downstream applications.

ISOLATE II BIOFLUIDS RNA COLUMN SPECIFICATIONS

Max. binding capacity	50 µg RNA
Max. column loading volume	650 µL
RNA size distribution	All sizes, including small RNA <200 nucleotides
A260/A280 ratio*	1.9-2.1
Typical RIN (RNA integrity number) †	>9
Max. amount of starting material:	
Biofluid (Blood/Saliva/Semen/Urine/CSF)	100 µL
Plasma/Serum	200 µL
Viruses	3 x 10^6 cells
	~20 mg mammalian tissue
	100 µL viral suspension or blood
	One swab
Biofluid in Cotton Swab	

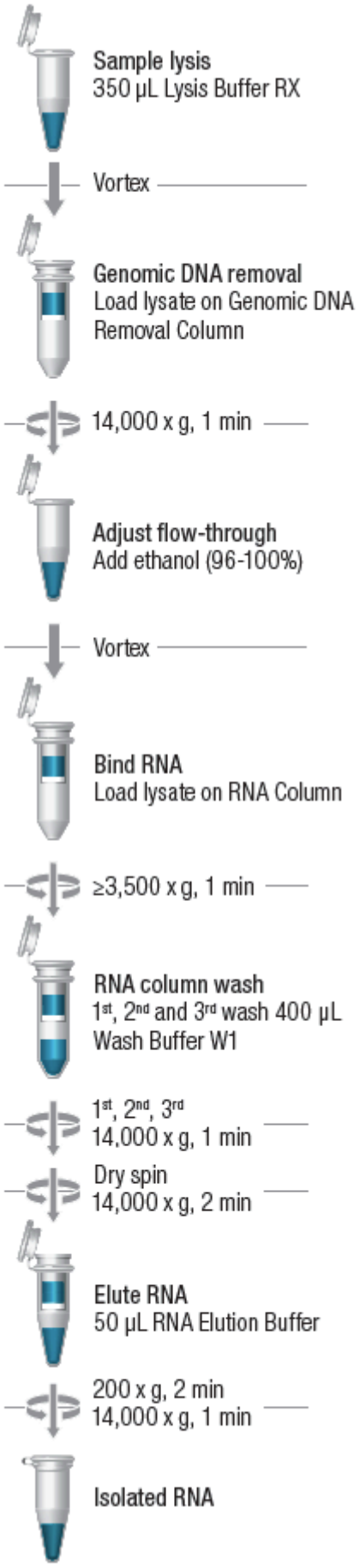
**Typically, the A_{260}/A_{280} ratio exceeds 1.9, indicating excellent RNA purity.*

† Agilent 2100 Bioanalyzer (RNA 6000 assay). RNA integrity is highly dependent on sample quality.

The following components are also included in the kit:

- Genomic DNA Removal Column for removing contaminating genomic DNA.
- DNase I (RNase-free) for eliminating genomic DNA contamination by on-column digestion or by digestion in solution (for the most sensitive applications).

Biofluid RNA Isolation



EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information.

The following may be supplied by the user:

- ß-mercaptoethanol (ß-ME)* (Optional for Lysis Buffer RX)
- 96-100% ethanol† (for Wash Buffer W1)
- Equipment for sample disruption and homogenization (see section 7.2). One or more of the following are required depending on chosen method:
  - o PBS (RNase-free) and trypsin
  - o Needle and syringe (both RNase free) o Mortar and pestle
  - o Rotor-stator homogenizer
  - o Liquid nitrogen
- Molecular biology grade water
- RNase-free microcentrifuge tubes (1.5 mL)
- Sterile RNase-free tips
- Sterile cotton swabs (for Nasal/Throat Swab Protocol)
- MS2 RNA (for Plasma/Serum Protocol)
- Benchtop microcentrifuge (capable of 14,000 x g)

**TCEP is also a suitable reducing agent instead of ß-ME.*
† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

IMPORTANT NOTES

The protocol steps are homogenization and lysis from different starting materials, genomic DNA removal, and purification of total RNA. The DNA removal and RNA purification protocols apply to all starting materials. Optional DNase I treatment protocols are provided.

• The collection contains the lysate preparation protocols for various starting materials; ensure you follow the specific protocol for your sample.
• The instructions for removing genomic DNA and purification of total RNA from all types of lysate are described in this protocol.
• For isolating RNA from plasma/serum samples, follow this dedicated protocol.
• This contains the optional protocols for additional DNase I treatment.

The ISOLATE II Biofluids RNA purification procedures can be performed at room temperature. Handle the eluted RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20°C for short-term or -80°C for long-term storage. When working with RNA samples in downstream applications, keep the RNA solution on ice.

Two types of spin columns are provided with this kit: the ISOLATE II Genomic DNA Removal Column (blue ring) and the ISOLATE II RNA Column (black ring). Ensure the correct column is used for each step of the procedure.

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g except where noted. Perform all centrifugation steps at room temperature.

Ensure that all solutions are at room temperature prior to use.

It is important to work quickly when purifying RNA (see hints and tips on working with RNA at www.bioline.com/isolate).

HANDLING AND STORING STARTING MATERIALS

RNA is not protected against digestion until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing reagents. Samples should be flash frozen in liquid nitrogen immediately and can be stored at -80°C for several months, or processed as soon as possible. Following disruption and homogenization in Lysis Buffer RX, samples can be kept at -80°C for up to one year, at 4°C for up to 24 hours or at room temperature for several hours. Frozen samples are stable for up to 6 months. Frozen samples in Lysis Buffer RX should be thawed slowly before starting the isolation of total RNA.

DISRUPTING AND HOMOGENIZING STARTING MATERIALS

For all RNA purification procedures, efficient disruption and homogenization of starting material is essential. To release all RNA contained in a sample, the total disruption of cell walls, plasma membranes and organelles must occur. Incomplete disruption results in reduced RNA yields. Homogenization reduces lysate viscosity following disruption and also facilitates efficient binding of RNA to the column membrane. Incomplete homogenization results in inefficient binding of RNA to the membrane and therefore reduced RNA yields.

Cells grown in a monolayer

Remove the cell culture medium completely. Incomplete removal of the medium will inhibit lysis of the cells and compromise the efficiency of RNA isolation. Wash with PBS and add Lysis Buffer RX immediately to the cell culture plate.

Cells grown in suspension

Centrifuge an appropriate number of cells and remove all supernatant by aspiration. Wash with PBS and lyse by adding Lysis Buffer RX.

Lifting of adherent cells with trypsin

To trypsinize adherent growing cells, first aspirate the cell culture medium. Add an equal volume of PBS to wash the cells and aspirate excess liquid. Add 0.1-0.3% trypsin in PBS to the washed cells. Incubate until the cells are detached. Add fresh, sterile culture medium and transfer cells to an appropriate tube (not supplied). Pellet cells by centrifugation for 10 min at 200 x g. Remove supernatant and add Lysis Buffer RX to the cell pellet.

Disruption using a mortar and pestle

An RNase-free mortar and pestle can be used in combination with liquid nitrogen to disrupt and lyse frozen or fibrous tissue samples, which are often solid. Grind the frozen tissue into a fine powder and add liquid nitrogen as necessary. It is important to ensure the sample does not thaw during or after grinding. After grinding, transfer tissue powder into a liquid nitrogen-cooled tube and allow the liquid nitrogen to evaporate. Add Lysis Buffer RX with reducing agent to the powdered tissue and mix immediately. Homogenize the sample with a nuclease-free 20 gauge (0.9 mm) syringe needle.

Disruption and homogenization using a rotor-stator homogenizer

Rotor-stator type tissue homogenizers can homogenize, disrupt and emulsify mammalian tissue samples in the presence of lysis buffer in seconds to minutes. Homogenization time depends on sample size and toughness. The spinning rotor disrupts and homogenizes the sample simultaneously by turbulence and mechanical shearing. Foaming can be minimized by keeping the rotor tip submerged. Select a suitably sized homogenizer: 5-7 mm diameter rotors can be used for homogenization in microcentrifuge tubes.

BUFFER PREPARATION AND PARAMETERS

Ensure 96-100% ethanol is available. Prepare the following:

Preparing Wash Buffer W1 with ethanol

Add 90 mL of 96-100% ethanol to Wash Buffer W1 Concentrate to give a final volume of 128 mL.

Note: Mark the label of the bottle to indicate ethanol was added. Store Wash Buffer W1 at room temperature (18-25°C).

Preparing Lysis Buffer RX with ß-mercaptoethanol (optional)

Optional: The use of ß-mercaptoethanol (ß-ME) in lysis is highly recommended for most mammalian tissues, particularly those known to have a high RNase content (e.g. pancreatic tissue). It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 µL of ß-ME (provided by the user) to each 1 mL of Buffer RX required. ß-ME is toxic and should be dispensed in a fume hood. Alternatively, Buffer RX can be used as provided.

Note: TCEP can also be used as an alternative reducing agent. Use TCEP at a final concentration of 10 mM within Lysis Buffer RX.

Preparing DNase I (RNase-free) (optional)

Optional on-column digestion: For each on-column reaction to be performed, prepare a mix of 15 µL of DNase I Solution and 100 µL of DNase I Reaction Buffer DRB. Mix gently by inverting a few times.

Optional in-solution digestion: In a microcentrifuge tube, mix together 10 µL of DNase I Reaction Buffer DRB, 2.5 µL of DNase I Solution and up to 87.5 µL of RNA solution. For lower starting volumes of RNA, bring the volume up to 100 µL using RNase-free water. Gently swirl tube to mix solution.

Note: Do not vortex the DNase I as the enzyme is particularly sensitive to mechanical denaturation. Dispense into aliquots to avoid excessive freeze-thawing. Store aliquots at -20°C.

Elution parameters

Elute RNA using RNA Elution Buffer (included). The standard elution protocol can be modified for different applications.

- To achieve high yield, perform two successive elution steps with an elution volume described in the individual protocol (90-100% recovery rate). You may elute into the same or a different microcentrifuge tube depending on your application.

- For both high-yield and high-concentration, elute with the standard elution volume. Then re-apply eluate onto the column for re-elution into the same microcentrifuge tube.

Always place eluted RNA on ice immediately to prevent degradation by RNases. For short-term storage freeze at -20°C but for long-term storage freeze at -80°C.

ELIMINATING GENOMIC DNA CONTAMINATION

For most applications, genomic DNA is undetectable in preparations of RNA using the ISOLATE II Biofluids RNA Kit. Genomic DNA contamination is efficiently removed by the Genomic DNA Removal Column. However, if the sample contains high amounts of genomic DNA, additional treatment can be performed with the supplied DNase I. Two alternative protocols are provided. The on-column digestion protocol efficiently removes genomic DNA sufficient for most applications. For highly sensitive applications e.g. probe-based real-time PCR, a DNase I digest in the eluate can be performed to remove even traces of contaminating DNA.

TROUBLESHOOTING GUIDE

Please see the full Bioline manual.
http://www.bioline.com/us/downloads/dl/file/id/3789/isolate_ii_biofluids_rna_kit_product_manual.pdf

Before start

Ensure Wash Buffer W1 is prepared (see guidelines).www.bioline.com

Materials

ISOLATE II Biofluids RNA Kit BIO-52086 by Bioline

Protocol

Genomic DNA Removal

Step 1.

Assemble an ISOLATE II Genomic DNA Removal Column (blue ring) with a Collection

Tube (provided).

NOTES

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Ensure you use the correct column: the Genomic DNA Removal Column has a **blue** ring.

Genomic DNA Removal

Step 2.

Apply up to 600 µL of lysate prepared from section 8 on to the column and centrifuge for 1 min at 14,000 x g.

DURATION

01:00:00

NOTES

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Note: Ensure that the entire lysate volume has passed into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional minute at 14,000 x g.

Genomic DNA Removal

Step 3.

Transfer the flow-through into a sterile RNase-free 1.5 mL microcentrifuge tube (user supplied) for

Total RNA Purification.

Genomic DNA Removal

Step 4.
The flow-through contains the RNA and should be stored on ice or at -20°C until the Total RNA Purification protocol is carried out.

Genomic DNA Removal

Step 5.
Discard the column with the bound genomic DNA.

Binding RNA to Column

Step 6.
To every 100 µL of flow-through, add 60 µL of 96-100% ethanol.

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Ensure you use the correct column: the ISOLATE II RNA Column has a **black** ring.

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For example, for 200 µL of flow-through, add 120 µL of ethanol.

Binding RNA to Column

Step 7.
Mix by vortexing.

Binding RNA to Column

Step 8.
Assemble an ISOLATE II **RNA Column** (black ring) with a provided Collection Tube.

Binding RNA to Column

Step 9.

Apply up to 600 µL of the ethanolic lysate onto the column and centrifuge for 1 min at ≥3,500 x g.

DURATION
00:01:00

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Note: Ensure the entire lysate volume has passed into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional minute at 14,000 x g.

Binding RNA to Column

Step 10.
Discard the flow-through.

Binding RNA to Column

Step 11.
Reassemble the spin column with its Collection Tube.

Binding RNA to Column

Step 12.
Depending on the lysate volume, repeat steps 9-11 as required.

Binding RNA to Column

Step 13.

Optional: The ISOLATE II Biofluids RNA Kit isolates Total RNA with minimal amounts of

genomic DNA contamination. However, for sensitive applications, an optional on-column DNA removal protocol is provided in Appendix A. DNase I treatment should be performed at this point in the protocol with the supplied DNase I. For highly sensitive applications, in-solution DNase I treatment can be performed (see Optional On-Column DNase I Treatment).

RNA Column Wash

Step 14.
Apply 400 µL of Wash Buffer W1 to the RNA Column and centrifuge for 1 min at 14,000 x g. (wash 1/3)

DURATION
00:01:00

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Note: Ensure the entire wash buffer volume has passed into the Collection Tube by inspecting the column. If the entire wash buffer volume has not passed through, centrifuge for an additional minute at 14,000 x g.

RNA Column Wash

Step 15.
Discard the flow-through and reassemble the spin column with the Collection Tube. (wash 1/3)

RNA Column Wash

Step 16.
Apply 400 µL of Wash Buffer W1 to the RNA Column and centrifuge for 1 min at 14,000 x g. (wash 2/3)

DURATION
00:01:00

RNA Column Wash

Step 17.

Discard the flow-through and reassemble the spin column with the Collection Tube. (wash 2/3)

RNA Column Wash

Step 18.

Wash column a third time by adding another 400 µL of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.

DURATION
00:01:00

RNA Column Wash

Step 19.

Discard flow-through and reassemble spin column with its Collection Tube.

RNA Column Wash

Step 20.

Centrifuge for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the

Collection Tube.

DURATION
00:02:00

RNA Elution

Step 21.

Place the RNA Column into a fresh 1.7 mL Elution Tube (supplied).

RNA Elution

Step 22.

Add 50 µL of RNA Elution Buffer to the column.

RNA Elution

Step 23.

Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column.

DURATION
00:02:00

RNA Elution

Step 24.

If the entire volume has not been eluted, spin column for an additional minute at 14,000 x g to elute the RNA.

DURATION
00:01:00

NOTES

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Note: For maximum RNA recovery, it is recommended to apply a second volume of RNA Elution Buffer and elute into the same microcentrifuge tube (repeat steps 22 - 24). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for high

concentration).

Storage of RNA

Step 25.

The isolated RNA can be stored at -20°C for a few days or at -80°C (recommended) for long- term storage.

Warnings

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Lysis Buffer RX contains guanidinium thiocyanate. This chemical is harmful in liquid form when in contact with skin or ingested. If the solution is allowed to dry, the powder is harmful if inhaled.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidinium salts. Reactive compounds and toxic gases can form. In the case of spillage, clean the affected area with a suitable laboratory detergent and water.

For detailed information, please consult the material data safety sheet (MSDS) available on our website at www.bioline.com.

Biofluids derived from all human and animal sources are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with biofluids.