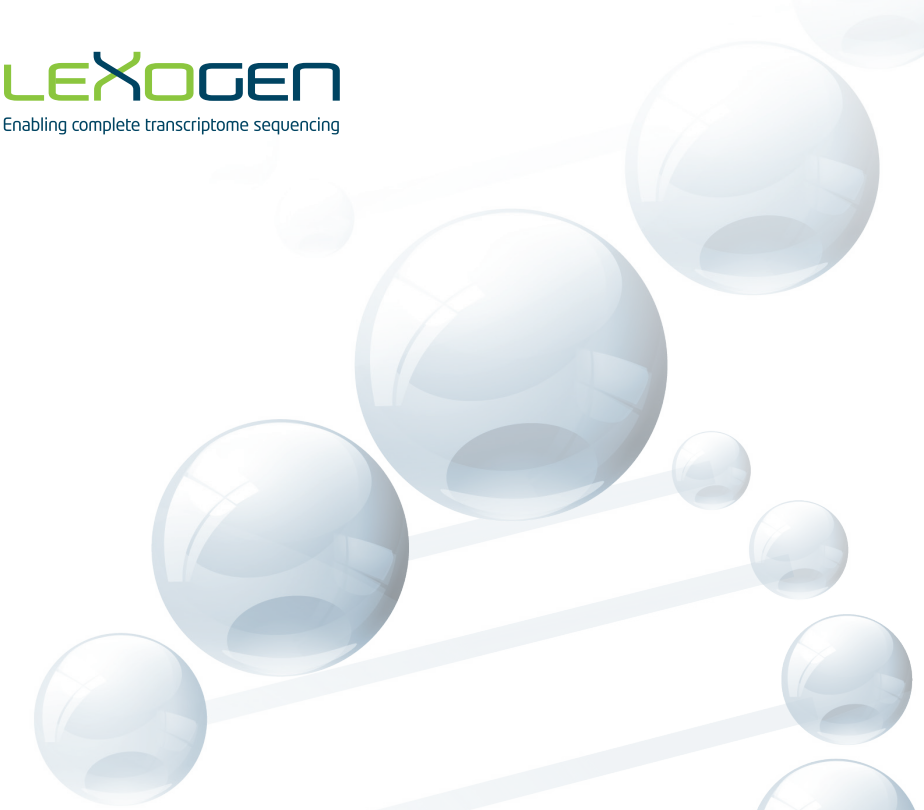
LEXOGEN

Enabling complete transcriptome sequencing



SPLIT

Fractions for pure RNA sequencing

Rapid Viral RNA/DNA Extraction Kit

User Guide

Catalog Numbers:
138 (SPLIT Rapid Viral RNA/DNA Extraction Kit)

138UG273V0100

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1. Overview

Lexogen's RNA Extraction Kits enable fast and highly efficient extraction of high quality, high purity RNA. The SPLIT Rapid Viral RNA/DNA Extraction Kit provides a streamlined protocol for isolation of RNA from liquid samples such as nasopharyngeal and buccal swabs. RNA can be recovered within 15 minutes using a quick and easy micro-spin column purification and can be directly used for molecular biology applications such as qRT-PCR, reverse transcription, and targeted RNA Sequencing.

SPLIT Rapid Viral RNA/DNA Extraction Kits are for RNA/DNA Extraction from liquid samples and can be used for isolation of viral RNA from a variety of viruses.

First, the sample is lysed in a highly chaotropic isolation buffer which facilitates effortless sample lysis, and guarantees complete RNase inhibition. In case any insoluble material is present after mixing, the lysate is cleared with a short additional centrifugation step.

The RNA is then precipitated onto a silica column by adding 0.55x volume of isopropanol to the sample lysed in Isolation Buffer. The column is then washed, dried and RNA is eluted using pre-warmed Elution Buffer. Isopropanol and ethanol for preparation of Wash Buffer have to be supplied by the user.

Please note that this kit is not designed to separate RNA from cellular DNA and both nucleic acids will be purified simultaneously from the sample. Samples containing epithelial cells, e.g., swab samples can be filtered or cleared of cells by centrifugation. The Kit does not contain an integrated DNA removal step. If needed, DNA can be removed following nucleic acid extraction. For demanding downstream analysis such as whole transcriptome sequencing that require highly pure DNA-free RNA preparations, an additional DNase I digest is required. Alternatively we recommend using the SPLIT RNA Extraction Kit (Cat. No. 008) for whole transcriptome NGS and full-length cDNA synthesis applications. For further information and protocol details please contact support@lexogen.com.

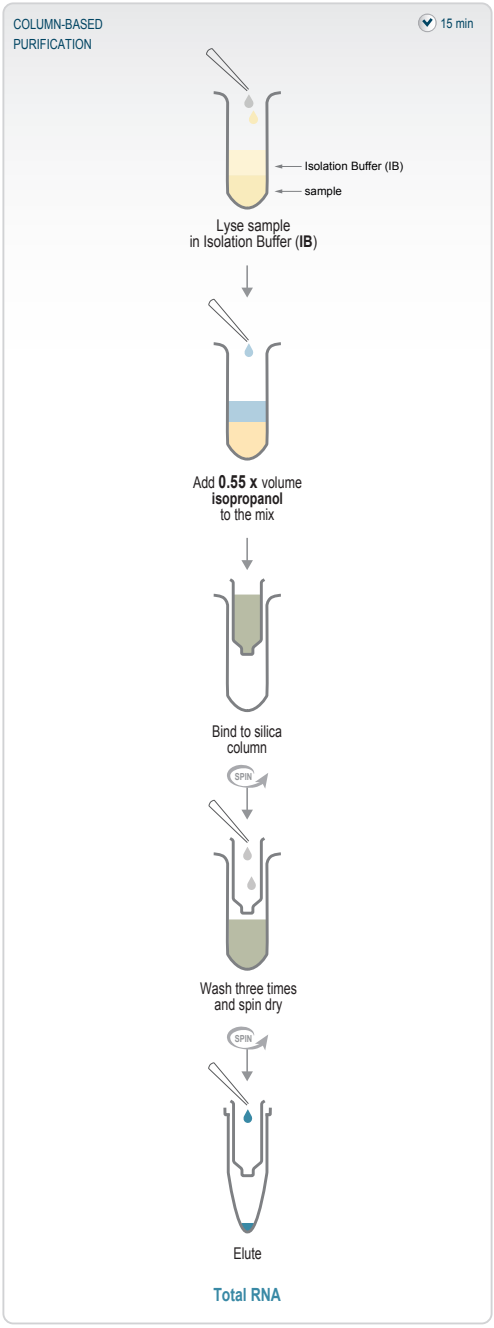

Figure 1. Schematic overview of the SPLIT Rapid Viral RNA/DNA Extraction workflow.

2. Kit Components and Storage Conditions

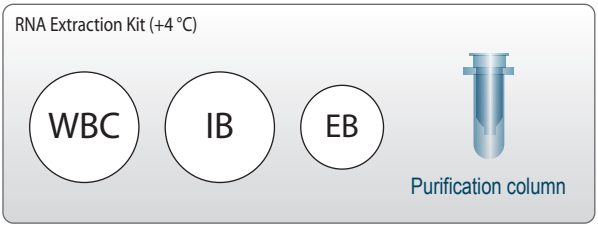

Figure 2. Schematic of kit components.

Kit Component	Label	Volume for 400 rxns	Storage
Isolation Buffer	**IB**	88 ml *	+4 °C
Wash Buffer Concentrate	**WBC**	132 ml [1,]*	+4 °C
Elution Buffer	**EB**	22 ml *	+4 °C
Purification columns	**Purification columns**	2 x 200	+4 °C or RT
Collection tubes	**Collection tubes**	2 x 200	+4 °C or RT

[1] Excluding ethanol (to be added by the user, mix in new bottle). * Including ≥10 % surplus.

The basic packaging unit of this kit is 400 reactions. The kit can be ordered and shipped in multiples of 400 reactions. Upon receiving the RNA Extraction Kit (Cat. No. 138) store it at +2 to +8 °C. Isolation Buffer (**IB**) is to be used at +4 °C. All other components should be equilibrated to room temperature before use. Check the contents of **IB** and **WBC** which may precipitate during shipping and storage. If a white precipitate is visible, incubate at 37 °C until buffer components dissolve completely.

Wash Buffer (**WB**) is prepared by diluting Wash Buffer Concentrate (**WBC**) with 4x volumes of absolute ethanol (EtOH abs.) per volume **WBC** prior to use. For best practice, it is recommended to measure the required volume of **WBC**, add the appropriate amount of EtOH abs., and decant into a sterile bottle with a wide opening (not provided) to avoid contamination of **WB** with non-sterile pipets. The table shows the volumes of **WBC** and EtOH abs. to be mixed for processing 25, 50, 100, 200, or 400 reactions (incl. 10 % surplus).

Reactions	Vol. WBC*	Vol. EtOH abs.*
25	8.25 ml	33 ml
50	16.5 ml	66 ml
100	33 ml	132 ml
200	66 ml	264 ml
400	132 ml	528 ml

* Including ≥10 % surplus.

3. User-Supplied Consumables and Equipment

Check to ensure that you have all of the necessary materials and equipment before beginning with the RNA extraction. All reagents, equipment, and labware must be free of nucleases and nucleic acid contamination.

Reagents

Reagent	Requirement for 400 rxns	Comment
Isopropanol	~ 97 ml	2-Propanol
Ethanol abs.	528 ml	EtOH abs., added to **WBC**

Equipment

- Benchtop centrifuge (12,000 x g, rotor compatible with 1.5 ml and 2.0 ml tubes).
- Calibrated single-channel pipettes for handling 10 µl to 1,000 µl volumes.
- Vortex mixer.
- UV-spectrophotometer to quantify RNA.

Labware

- Sterile glass or plastic ware (measuring cylinders, bottles).
- Suitable pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml and 2.0 ml tubes with cap, low binding, certified ribonuclease-free.
- Benchtop cooler or ice pellets in ice box (for short-term storage of RNA).

Optional Equipment & Solutions

- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer).
- Agarose gels, dyes, and electrophoresis rig (for RNA quality control).
- DNA-ExitusPlus (AppliChem GmbH).
- RNaseZap.
- RNase inhibitor.

The complete set of materials, reagents, and labware for quality control is not listed.

4. Guidelines

Safety Information

- The Isolation Buffer (**IB**) contains guanidine isothiocyanate, an irritant, which upon protocol completion is also present in flow-through and wash fractions. This chemical is harmful when in contact with the skin, inhaled, or ingested. Do not add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidine isothiocyanate, as reactive compounds and toxic gases are formed.
- Solutions containing isopropanol or ethanol are considered flammable. Use appropriate precautions when using these chemicals.
- For your protection, always wear a laboratory coat, gloves, and safety glasses when handling chemicals.
- Dispose buffers and chemicals in appropriate waste containers.
- Consult the appropriate Material Safety Data Sheets (MSDS) at www.lexogen.com, and contact your Environmental Health and Safety department for proper work and disposal guidelines.

General

- Ensure that adequate volumes of all reagents and the necessary equipment are available before beginning the protocol.
- Before you start, check all solutions for the formation of precipitate and if necessary, incubate at 37 °C until buffer components dissolve completely.
- Unless indicated differently, centrifugation should be performed at 18 °C to increase reproducibility. If a refrigerated centrifuge is not available, centrifugation can be carried out at room temperature (20 - 25 °C). Results may be negatively impacted if the protocol is performed at temperatures outside of 18 - 25 °C.
- Perform all pipetting steps with calibrated pipettes and always use fresh tips. Pipette carefully to avoid foaming as the Isolation Buffer (**IB**) contains detergents.

RNA Handling

- RNases are ubiquitous, and special care should be taken throughout the procedure to avoid RNase contamination.
- Use commercial ribonuclease inhibitors (i.e., RNasin, Promega Corp.) to maintain RNA integrity when storing samples.
- Use a sterile and RNase-free workstation or laminar-flow hood if available. Please note that RNases may still be present on sterile surfaces, and that autoclaving does not completely eliminate RNase contamination.
- Before starting an RNA extraction, clean your work space, pipettes, and other equipment with RNase removal spray (such as RNaseZap, Ambion Inc.) as per the manufacturer's instructions.
- Protect all reagents and your RNA samples from RNases on your skin by wearing a clean lab coat and fresh gloves. Change gloves after making contact with equipment or surfaces outside of the RNase-free zone.
- Avoid speaking above opened tubes. Keep reagents closed when not in use to avoid airborne RNase contamination.

5. Detailed Protocol

Rapid Extraction of RNA

Preparation

	Total RNA	Temperature
Isolation Buffer (IB)	200 µl	RT
Isopropanol	220 µl	RT
Wash Buffer (WB)	1,650 µl	RT
Elution Buffer (EB)	10 - 50 µl	RT
Purification column	1	RT
Collection tube	1	RT
2.0 ml tube	-	RT
1.5 ml tube	1	
Centrifuge		18 °C
Vortex mixer		

Caution: Discard waste containing guanidine isothiocyanate according to applicable Health and Safety regulations.

Liquid samples are mixed with Isolation Buffer (IB) for lysis. In case any insoluble material is present after mixing, it is suggested to clear the lysate with a short additional centrifugation step. Isopropanol is added to sample/IB, mixed and loaded onto the column. After washing, the RNA is eluted in Elution Buffer (EB) and can be used for further applications.

ATTENTION: Wash Buffer (**WB**) needs to be prepared from Wash Buffer Concentrate (**WBC**), please refer to p.6 for details.

REMARK: Centrifugation times may need to be increased if the sample does not pass the filter completely. In case a refrigerated centrifuge is not available, centrifugation steps can be performed at room temperature. Pre-warm Elution buffer (EB) or Storage Buffer (SB) at 70 °C to increase recovery. Storage Buffer is not provided with the RNA Extraction Kit (see p.14 for details).

1. Add 200 µl Isolation Buffer (**IB**) to 200 µl of the liquid sample and mix by vortexing. **OPTIONAL:** In case insoluble material is present, clear by centrifuging for 1 minute at 12,000 x g at 18 °C. Transfer the cleared lysate to a new tube and proceed.
2. Add 220 µl of Isopropanol and mix by pipetting. **ATTENTION:** Do not centrifuge after this step.
3. Place the purification column into the collection tube. Transfer up to 700 µl of the sample/**IB**/Isopropanol mixture to the column, close the lid gently.

4	Centrifuge for 20 seconds at 12,000 x g at 18 °C and discard the contents of the collection tube.
5	Apply 500 µl of Wash Buffer (**WB**) to the column and centrifuge for 20 seconds at 12,000 x g at 18 °C. Empty the collection tube. Repeat this step twice for a total of three washes.
6	Centrifuge for 1 minute at 12,000 x g at 18 °C. **ATTENTION:** This step is essential to remove all traces of ethanol.
7	Discard the collection tube and place the purification column in new 1.5 ml tube. **ATTENTION:** Avoid carryover of ethanol.
8	Add 10 - 50 µl of the pre-warmed Elution Buffer (**EB**) or Storage Buffer (**SB**) to the column and incubate for 1 minute at room temperature.
9	Centrifuge for 1 minute at 12,000 x g at 18 °C to elute purified RNA.
10	The RNA is now ready for quality control and downstream applications. **OPTIONAL:** RNase inhibitor (not included) can be added. Note that the RNase inhibitor might absorb at 230 nm, therefore use buffer with RNase inhibitor added as blank for OD measurements.

6. Short Procedure

Rapid Extraction of RNA

ATTENTION: All centrifugation steps are at 12,000 x g and 18 °C unless otherwise mentioned!

15 min Column-based RNA Extraction

	Lysis of Liquid Samples
☐	Add 200 µl Isolation Buffer (**IB**) to 200 µl of the liquid sample and mix by vortexing. **OPTIONAL:** In case insoluble material is present, clear by centrifuging for 1 minute at 12,000 x g at 18 °C. Transfer the cleared lysate to a new tube and proceed.
	Column Loading Total RNA
☐	Add 220 µl Isopropanol and mix by pipetting. **ATTENTION:** Do not centrifuge after this step.
☐	Place the purification column into the collection tube. Transfer up to 700 µl of the sample/**IB**/Isopropanol mixture to the column, close the lid gently.
☐	Centrifuge for 20 seconds at 12,000 x g at 18 °C and discard the contents of the collection tube.
	Column Washing
☐☐☐	Apply 500 µl **WB** and centrifuge for 20 sec. Empty collection tube. Repeat this step twice for a total of three washes.
☐	Centrifuge for 1 min to spin dry column. **ATTENTION:** This step is essential to remove all traces of ethanol.
	Elution
☐	Place purification column in a 1.5 ml tube.
☐	Pre-warm **EB** for 5 min at 70 °C. **OPTIONAL:** Storage Buffer (**SB**) can be used (see Appendix C, p.14)
☐	Apply 10 - 50 µl of pre-warmed **EB** (or **SB**), incubate for 1 min at RT.
☐	Centrifuge for 1 min.
☐	**OPTIONAL:** Add RNase inhibitor (not included).

REMARK: Repeat the centrifugation or increase centrifugation time if the sample did not fully pass through the filter completely.

7. Appendix A: Sample Input and Extraction Efficiencies

Results and performance were intensively characterized using the SPLIT RNA Extraction Kit (Cat. No. 008) or the SPLIT for Blood Kit (Cat. No. 099). These kits contain Phase Lock Gel tubes to facilitate phase separation during Phenol / Chloroform extraction, for detailed specifications of the SPLIT RNA Extraction Kit, please refer to 008UG005.

If immediate RNA extraction is not possible, liquid samples can be either flash-frozen with liquid nitrogen and mixed with IB and stored at -80 °C (recommended) or -20 °C.

The maximum binding capacity of the purification column is 10 µg RNA, which should not be exceeded for optimal results.

8. Appendix B: RNA Quality Control

RNA Integrity

The integrity of an RNA sample can be assessed with a variety of methods, e.g., using a microfluidics assay such as the RNA6000 series for the 2100 Bioanalyzer (Agilent Technologies Inc.) or denaturing agarose gel electrophoresis if such a device is not available. Most microfluidics platforms will carry out an automated peak analysis and generate a quality score (RIN or RQN) in addition to the 28S/18S rRNA ratio. However, the Viral RNA/DNA Extraction kit co-isolates DNA, hence a RIN may not be determined unless the DNA is removed.

Potential Contaminants

RNA samples should be free of salts, metal ions, and organic solvents, which can be carried over from the RNA extraction. Several sources of contamination can be detected with a UV-Vis spectrophotometer. An acceptably pure RNA sample should have an A260/A280 ratio between 1.8 and 2.1. The A260/A230 ratio should also be greater than 1.8. Several common contaminants including proteins, chaotropic salts and phenol absorb strongly between 220 and 230 nm and can often be identified as peaks in this region. Contamination with any of these generates a lower A260/230 ratio.

Genomic DNA Contamination

The SPLIT Rapid Viral RNA/DNA Extraction Kit (Cat. No. 138) does not contain a genomic DNA (gDNA) removal step. For demanding downstream analyses that require highly pure DNA-free RNA preparations, an additional DNase I digest is required. For further information and protocol details please contact support@lexogen.com. gDNA is indistinguishable from RNA on a spectrophotometer, and many of the dyes used in RNA microfluidics assays stain single-stranded nucleic acids much more intensely than double-stranded. Hence, low to moderate amounts of gDNA may not be readily visible with an RNA-specific microfluidics assay. We highly recommend examining all RNA samples on a denaturing agarose gel or using a fluorometric assay with DNA- and RNA-specific dyes to check samples for DNA contamination. On an agarose gel, gDNA can appear as either a dark mass which remains in the slot if relatively intact or as a high molecular weight smear if it has been sheared during extraction. The SPLIT RNA Extraction Kit (Car. No. 008) was designed for minimizing the gDNA content in the RNA sample.

9. Appendix C: RNA Storage

After extraction, RNA can be stored in Elution Buffer (**EB**, 10 mM Tris-HCl pH 7.0) at -20 °C or -80 °C. This minimal buffer stabilizes the pH without any other components that might interfere with downstream applications. When eluting in **EB** we highly recommend the addition of RNase inhibitors to block any accidentally introduced RNases.

The Storage Buffer (**SB**, 10 mM Tris-HCl pH 7.0, 10 mM DTT and 0.1 mM EDTA) is not supplied with this product. In case Storage Buffer is required, please contact info@lexogen.com. **SB** can be used for intermediate storage of the RNA at -20 °C or -80 °C. DTT (antioxidant) and EDTA (chelating agent) both minimize the threat of RNA degradation, especially at non-freezing conditions. For long-term storage, we recommend keeping aliquots of the RNA as NaAc / ethanol precipitate at -80 °C to avoid accidental RNase contamination as well as RNA degradation due to freeze / thaw cycles.

We suggest checking the RNA quality after extended periods of storage for changes in integrity and quantity e.g., on a microfluidics system.

10. Appendix D: Revision History

Publication No. / Revision Date	Change	Page
138UG273V0100 June 18, 2020	Initial Release.	

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Associated Products:

008 (SPLIT RNA Extraction Kit)
099 (SPLIT RNA Extraction Kit for Blood)

SPLIT Rapid Viral RNA/DNA Extraction Kit · User Guide

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