

The background features a series of blue, semi-transparent spheres of varying sizes, connected by thin, light blue lines that create a network-like structure. The spheres have a glossy, reflective surface, and the lines are slightly curved, giving the overall design a sense of movement and connectivity.

LEXOGEN

The RNA Experts

SPLIT[™]

Pure RNA for all your experiments

One-step FFPE RNA Extraction Kit User Guide

Catalog Number:
236 (SPLIT One-Step FFPE RNA Extraction Kit)

236UG788V0100

FOR RESEARCH USE ONLY. NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE.

INFORMATION IN THIS DOCUMENT IS SUBJECT TO CHANGE WITHOUT NOTICE.

Lexogen does not assume any responsibility for errors that may appear in this document.

PATENTS AND TRADEMARKS

SPLIT is a trademark of Lexogen. Lexogen is a registered trademark (EU, CH, US, CN, AU, NO, BR). Qubit™ and RNaseZap™ are registered trademarks of Ambion, Inc., Bioanalyzer®, Fragment Analyzer™, TapeStation™ are trademarks of Agilent Technologies, Inc., DNA-ExitusPlus™ is a trademark of AppliChem GmbH, Inc., ThermoMixer® is a registered trademark of Eppendorf AG. All other brands and names contained in this user guide are the property of their respective owners.

Lexogen does not assume responsibility for violations or patent infringements that may occur with the use of its products.

LIABILITY AND LIMITED USE LABEL LICENSE: RESEARCH USE ONLY

This document is proprietary to Lexogen. The SPLIT One-step FFPE RNA Extraction Kits are intended for use in research and development only. They need to be handled by qualified and experienced personnel to ensure safety and proper use. Lexogen does not assume liability for any damage caused by the improper use or the failure to read and explicitly follow this user guide. Furthermore, Lexogen does not assume warranty for merchantability or suitability of the product for a particular purpose.

The purchase of the product is subject to Lexogen general terms and conditions (www.lexogen.com/terms-and-conditions/) and does not convey the right to resell, distribute, further sublicense, repackage, or modify the product or any of its components. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way without the prior written consent of Lexogen.

For information on purchasing additional rights or a license for use other than research, please contact Lexogen.

WARRANTY

Lexogen is committed to providing excellent products. Lexogen warrants that the product performs to the standards described in this user guide until the expiration date. Should this product fail to meet these standards due to any reason other than misuse, improper handling, or storage, Lexogen will replace the product free of charge or issue a credit for the purchase price. Lexogen does not provide any warranty if product components are replaced with substitutes.

Under no circumstances shall the liability of this warranty exceed the purchase price of this product.

LITERATURE CITATION

When describing a procedure for publication using this product, please refer to it as the SPLIT One-step FFPE RNA Extraction Kit.

We reserve the right to change, alter, or modify any product without notice to enhance its performance.

CONTACT INFORMATION

Lexogen GmbH

Campus Vienna Biocenter 5

1030 Vienna, Austria

www.lexogen.com

E-mail: support@lexogen.com

Support

E-mail: support@lexogen.com

Tel. +43 (0) 1 3451212-41

Fax. +43 (0) 1 3451212-99

Table of Contents

1. Overview.	4
2. Kit Components and Storage Conditions	6
3. User-Supplied Consumables and Equipment	7
4. Detailed Protocol	8
4.1. FFPE Tissue Preparation and Lysis	8
4.2. RNA Purification	10
5. Short Procedure.	12
6. Appendix A: Sample Input.	13
7. Appendix B: RNA Quality Control and RNA yield	13
8. Appendix C: Typical Results	15
9. Appendix D: Revision History.	16

1. Overview

The SPLIT One-step FFPE RNA Extraction Kit enables rapid and efficient extraction of high quality, high purity RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. The obtained RNA is ideal for seamless library preparation for Next Generation Sequencing (NGS) and other downstream applications such as RT-qPCR.

First, the FFPE sample undergoes optimized decrosslinking followed by the tissue lysis and paraffin and detergent removal. After short centrifugation, the lower aqueous phase is transferred into a fresh tube and treated with DNase to remove genomic DNA. The lysate is then subjected to the innovative One-step purification technology. Samples are loaded on the purification resin, which retains cellular debris and impurities, while RNA migrates freely through the matrix into the flow-through. The method requires only a single quick centrifugation step, cutting down significantly on both overall and hands-on time when compared to traditional silica methods. As the conventional bind-wash-elute procedure is replaced by a One-step purification, it also leads to a decrease in plastic waste. Furthermore, the protocol utilizes fewer hazardous reagents.

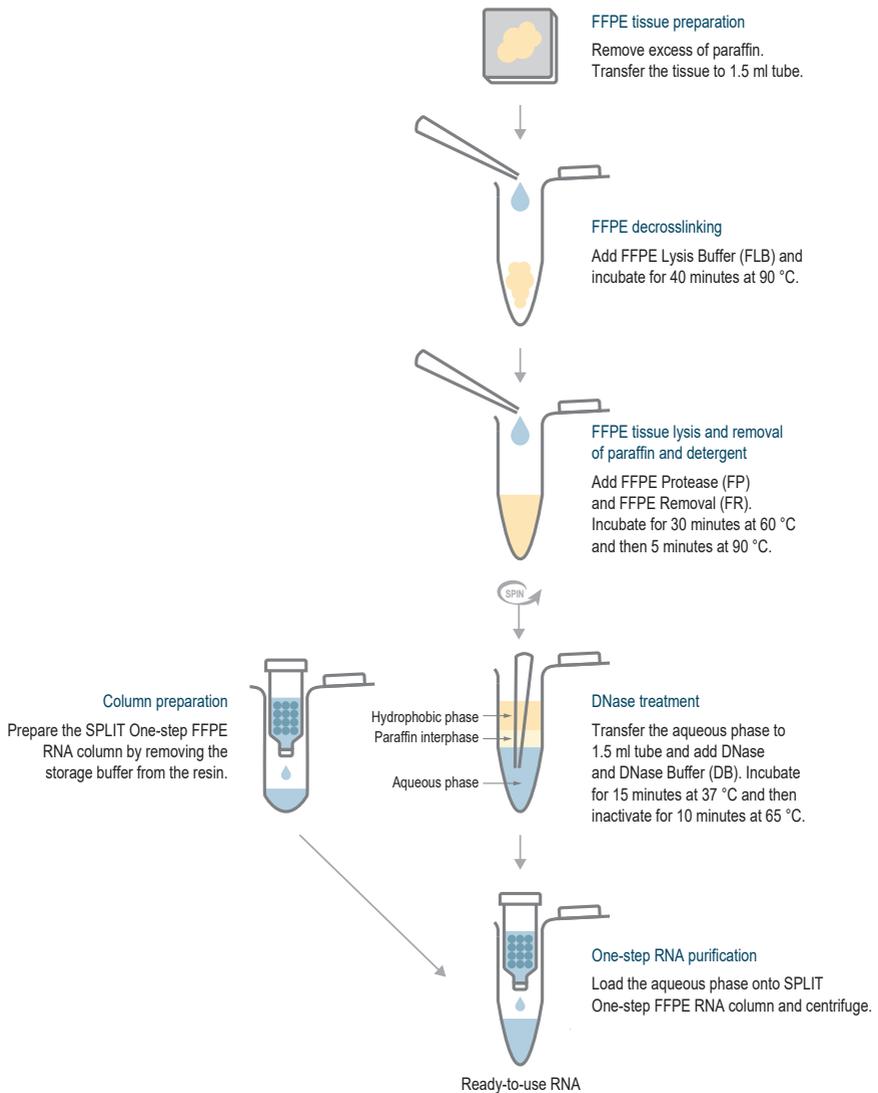


Figure 1. Schematic overview of the SPLIT One-step FFPE RNA Extraction Kit workflow. The optimized decrosslinking step, lysis, and detergent, paraffin removal followed by One-step RNA purification provide a fast and convenient solution for RNA extraction from challenging FFPE samples.

2. Kit Components and Storage Conditions

Kit Component	Label	Volume			Storage
		24 extractions	96 extractions	384 extractions	
FFPE Lysis Buffer	FLB ○	2,904 µl*	11,616 µl*	4x 11,616 µl*	🔒 +4 °C
FFPE Protease	FP ●	264 µl*	1,056 µl*	4x 1,056 µl*	🔒 +4 °C
FFPE Removal	FR	5,280 µl*	21,120 µl*	4x 21,120 µl*	🔒 +4 °C
DNase	D ●	26.4 µl*	105.6 µl*	4x 105.6 µl*	❄️ -20 °C
DNase Buffer	DB ●	264 µl*	1,056 µl*	4x 1,056 µl*	🔒 +4 °C
Low-TE Buffer	TB ●	1,200 µl	1,200 µl	4x 1,200 µl	🔒 +4 °C
FFPE SPLIT RNA columns		24	96	4x 96	🔒 +4 °C

*including ≥10% surplus

Following solutions contain hazardous components:

FR:  GHS07

FP ●:     GHS05 GHS07 GHS08 GHS09

Wear suitable personal protective equipment (PPE). Handle in accordance with good industrial hygiene and safety practice. Read the safety data sheet (SDS) for more information. Request SDS at www.lexogen.com/request-sds-form.

Upon receiving the SPLIT One-step FFPE RNA Extraction Kit, store the Box 1 (**FLB ○**, **FP ●**, **FR**, **DB ●**, **TB ●**, **FFPE SPLIT RNA columns**) at +4 °C and the Box 2 (**D ●**) at -20 °C. All components of Box 1 except for **FP ●** can be also stored at stable room temperature (15-25 °C).

Before use, check the contents of **FLB ○**, which may precipitate during storage at +4 °C. If a precipitate is visible or the content appears turbid, leave the buffer at room temperature until buffer components dissolve completely or heat it up to 56 °C to speed up the dissolving process.

FR can become solid when stored at +4 °C. Leave it at room temperature for 30 - 90 minutes to become liquid again or heat it up to 56 °C to speed up the dissolving process.

Keep DNase **D ●** in a benchtop cooler or on ice when in use. DNase is sensitive to physical inactivation – mix gently by pipetting or slowly reverting a tube.

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.

ATTENTION: Before starting this protocol, please read the [General Guidelines for Lexogen Kits](#), which are available online. These provide a detailed overview of RNA and kit component handling, as well as general RNA input requirements.

Equipment

- Benchtop centrifuge (rotor compatible with 1.5 ml and 2.0 ml tubes, minimum speed 19,000 x g).
- Calibrated single-channel pipettes for handling 1 µl to 200 µl volumes.
- Vortex mixer.
- ThermoMixer.
- UV-spectrophotometer to quantify RNA.
- Benchtop fluorometric assays, e.g., Qubit™.

Labware

- 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 and DNase).

Optional Equipment & Solutions

- Column Adapters for Plate Centrifuges (Cat. No. 239) enable the spinning of columns in a plate centrifuge with the swing-out rotor, resulting in the formation of a flat resin surface. Additionally, the adapters can hold up to 24 tubes of 1.5 or 2 ml each, allowing for the processing of multiple samples simultaneously and conveniently.
- Automated microfluidic electrophoresis station (Agilent Technologies 2100 Bioanalyzer, Fragment Analyzer, TapeStation).
- 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. Detailed Protocol

4.1. FFPE Tissue Preparation and Lysis

Before use, check **FLB** ○ for precipitation, which may occur during storage at +4 °C. If a precipitate is visible or the content appears turbid, leave the buffer at room temperature until buffer components dissolve completely or heat it up to 56 °C to speed up the dissolving process. **FR** can become solid when stored at +4 °C. Leave it at room temperature for 30 - 90 minutes to become liquid again or heat it up to 56 °C to speed up dissolving.

Preparation

FFPE Tissue Transfer and Initial Preparation of Columns	FFPE Tissue Decrosslinking	FFPE Tissue Lysis and Removal of Paraffin and Detergent
FFPE SPLIT RNA columns stored at +4 °C/ RT	FLB ○ stored at +4 °C/ RT, equilibrate to RT	FP ● stored at +4 °C FR stored at +4 °C/RT, equilibrate to RT
FFPE tissue samples Microtome RNase-free 1.5 ml and 2 ml tubes	ThermoMixer 90 °C, 1,400 rpm	ThermoMixer 60 °C, 1,400 rpm ThermoMixer 90 °C, 1,400 rpm

FFPE Tissue Transfer and Initial Preparation of Columns

Thoroughly mix the resin in the **FFPE SPLIT RNA column** by vigorously vortexing it upside down. Gently tap or flick the column to eliminate any air bubbles. Transfer the column into 2 ml tubes and allow the resin to sit for a minimum of 60 minutes before use.

NOTE: This step can be performed upon receipt of the kit or a day in advance. Then the columns should be stored in an upright position until use.

1

Prepare 1 – 15 mg FFPE tissue per sample or sections with a surface area of 100 – 800 mm². The recommended thickness of the sections is 10 µm. (usually 1 - 3 sections are sufficient). For more information, please refer to Appendix A, p.13.

2

ATTENTION: Trim excess of paraffin as it might negatively influence protocol efficiency and reduce RNA yield.

3

Place the FFPE sections in a 1.5 ml tube.

FFPE Decrosslinking

- 4 Add 110 µl FFPE Lysis Buffer (**FLB** ○) to each sample.
-

Incubate for 40 minutes at 90 °C and 1,400 rpm in a ThermoMixer. Ensure that the tissue is at the bottom of the tube and fully covered with the liquid.

- 5 **NOTE:** Incubation time can be reduced to 30 minutes when dealing with highly degraded samples. Be aware that reduced incubation time can, however, result in decreased RNA yield.
-

- 6 Spin down briefly and let the samples cool down at room temperature (~5 minutes)

NOTE: Do not place the tubes on ice to cool down.

FFPE Tissue Lysis and Removal of Paraffin and Detergent

- 7 Add 10 µl FFPE Protease (**FP** ●) and 200 µl FFPE Removal (**FR**) to each sample and vortex briefly.
-

ATTENTION: Do not prepare mastermix from **FP** ● and **FR** as the solutions do not mix.

Lyse the samples in a ThermoMixer for 30 minutes at 60 °C and 1,400 rpm.

- 8 **NOTE:** You can proceed with the conditioning of **FFPE SPLIT RNA columns** during the incubation time.
-

- 9 Incubate in a ThermoMixer for 5 minutes at 90 °C and 1,400 rpm to inactivate the **FP** ●.
-

- 10 Let the samples cool down for 3 minutes at room temperature.
-

4.2. RNA Purification

Conditioning of Columns	DNase Treatment	One-step RNA purification
FFPE SPLIT RNA columns equilibrate to RT	D ● stored at -20 °C DB ● stored at +4 °C /RT	Prepared FFPE SPLIT RNA columns TB ● stored at +4 °C /RT
Centrifuge at RT RNase-free 1.5 ml and 2 ml tubes	Centrifuge at RT RNase-free 1.5 ml tubes ThermoMixer 37 °C, 300 rpm ThermoMixer 65 °C, 300 rpm	Centrifuge at RT

Conditioning of FFPE SPLIT RNA Columns

- 11 Remove the bottom closure and loosen the lid of the **FFPE SPLIT RNA column** with a 180° turn (half-turn) to the right.
- 12 Place the column back into a 2 ml tube and centrifuge for 1 minute at 1,000 x g.

Discard the collection tube with the flow-through and place the column in a new 1.5 ml tube.

- 13 **NOTE:** When using a fixed rotor centrifuge, the resin in columns may be slightly tilted (~30° based on the rotor), which does not impact the column performance. Alternatively, a column adapter designed for a plate centrifuge with a swing-out rotor can be used to achieve a flat purification resin surface.

DNase Treatment

Centrifuge the samples from step ¹⁰ for 4 minutes at maximum speed (minimum 19,000 x g) for phase separation (upper phase: hydrophobic, interphase: paraffin, lower phase: aqueous)

- 14 **ATTENTION:** If the phases are not distinctly separated, centrifuge for an additional 6 minutes.
ATTENTION: If the lower aqueous phase appears cloudy due to remaining paraffin, reheat the sample for max 3 minutes at 60 °C and centrifuge again for 4 minutes at maximum speed.

- 15 Transfer the lower aqueous phase (~ 80 µl) to a new 1.5 ml tube.

- 16 Prepare a DNase mastermix containing 1 µl DNase (D ●) and 10 µl DNase Buffer (DB ●) per reaction. Mix the mastermix gently and keep it on ice.

- 17 Add 11 μ l of the DNase mastermix to each sample, mix by gently inverting the tube several times and spin down briefly.

- 18 Perform DNase digestion for 15 minutes at 37 °C and 300 rpm in a ThermoMixer.

- 19 Heat-inactivate DNase for 10 minutes at 65 °C and 300 rpm.

- 20 Briefly spin down and let samples cool down to room temperature.

One-step RNA Purification

- 21 Load the sample in the center of the prepared FFPE SPLIT RNA column. Pipet slowly, dropwise and keep the pipet in a vertical position.
ATTENTION: Make sure not to touch the resin surface.

- 22 Close the column lid and then loosen the lid by a half-turn to the right.

- 23 Centrifuge for 1 minute at 1,000 x g.

The flow-through contains purified RNA ready for quality control (Appendix B, p.13) and downstream applications. Elution volume typically ranges from ~ 80 -100 μ l.

- 24 **ATTENTION:** Use provided **TB** ● as a blank for UV-spectrometer, not water.
NOTE: Purified RNA is in low-TE buffer which does not impact downstream applications, including RNA-Seq.
-

5. Short Procedure

1 h 25 min FFPE Tissue Preparation and Lysis

FFPE Tissue Preparation

- Homogenize **FFPE SPLIT RNA columns** by vortexing upside down and remove any air bubbles. Place the columns into 2 ml tubes and let sit for a minimum of 60 minutes.
- Prepare 1 – 15 mg FFPE tissue per sample or sections with a thickness of up to 10 µm and a surface area of 100 - 800 mm².
- Place the FFPE sample into a 1.5 ml tube.

FFPE Decrosslinking

- Add 110 µl **FLB** to each sample.
- Incubate for 40 minutes at 90 °C and 1,400 rpm.
- Spin down briefly and cool down to room temperature.

FFPE Tissue Lysis and Removal of Paraffin and Detergent

- Add 10 µl **FP** and 200 µl **FR** to each sample and briefly vortex.
- Incubate for 30 minutes at 60 °C and 1,400 rpm.
- Incubate for 5 minutes at 90 °C and 1,400 rpm.
- Cool down for 3 minutes at room temperature.

50 min RNA Purification

Conditioning of FFPE SPLIT RNA Columns

- Loosen the cap of **FFPE SPLIT RNA column** by one half-turn and remove the bottom closure.
- Place the column into a 2 ml tube and centrifuge for 1 minute at 1,000 x g.
- Place the column into a fresh 1.5 ml tube.

DNase Treatment

- Centrifuge the samples for 4 minutes at maximum speed (minimum 19,000 x g)
- Transfer the lower aqueous phase to a new 1.5 ml tube.
- Prepare a DNase master mix: 1 µl **D** and 10 µl **DB** per reaction. Mix gently and keep on ice.
- Add 11 µl of the DNase mastermix to each sample, mix gently.
- Incubate for 15 minutes at 37 °C and 300 rpm.
- Incubate for 10 minutes at 65 °C and 300 rpm.
- Briefly spin down and cool down to room temperature.

One-step RNA Purification

- Slowly, dropwise apply the sample to the prepared **FFPE SPLIT RNA column**.
- Close the column cap and then loosen the cap by one half-turn.
- Centrifuge for 1 minute at 1,000 x g. The flow-through contains purified RNA.

6. Appendix A: Sample Input

RNA extraction from FFPE samples can be challenging due to the degraded nature of FFPE samples, the high variability introduced by block preparation techniques, storage conditions, and tissue type.

We recommend using 1-15 mg of FFPE tissue as an input amount. Alternatively, sections of 100 mm² to 800 mm² of tissue surface area, usually 1- 3 sections in total, and 10 µm in thickness are recommended. Please refer to the table below for more details:

Sections Thickness	Maximum Input
10 µm	1 slice with 800 mm ²
	2 slices with 400 mm ²
	3 slices with 260 mm ²
5 µm	1 slice with 1,600 mm ²
	2 slices with 800 mm ²
	3 slices with 530 mm ²
	4 slices with 400 mm ²
20 µm	1 slice with 400 mm ²
	2 slices with 200 mm ²
	3 slices with 130 mm ²
	4 slices with 100 mm ²

FFPE specimens should be stored at 2 °C to 8 °C or preferably at -20 °C to -70 °C for prolonged periods. It is crucial to remove excess paraffin from the samples whenever possible. Samples with high paraffin content may exhibit lower lysate and flow-through volumes, resulting in reduced RNA yield as paraffin excess inhibits flow through the purification resin.

If the input amount is below recommended, decreasing the FFPE lysis buffer volume from 110 µl to 70 µl can increase the final RNA concentration. However, this adjustment may lead to a lower overall yield and less convenient handling. Furthermore, a minimum of 50 µl of aqueous phase must be applied to the FFPE SPLIT RNA column to ensure a good flow inside the column. When decreasing the volume of the FFPE lysis buffer, ensure to scale down all solutions accordingly.

7. Appendix B: RNA Quality Control and RNA yield

The quality and the yield of extracted RNA, as well as its performance in downstream applications, are significantly influenced by the preparation process and storage conditions of FFPE samples and also by tissue type. Larger biopsies may not be adequately and uniformly pre-

served, consequently showing higher tissue degradation. Delaying the fixation of tissue can result in lower yield and RNA degradation. Long-time stored FFPE samples may exhibit increased RNA fragmentation, resulting in lower yield and RNA quality, and thus poor performance in downstream applications.

RNA Integrity and Purity

RNA extracted from FFPE samples usually exhibits low integrity due to the FFPE block preparation process and long-term storage. We recommend using microfluidics assays such as Fragment analyzer, TapeStation, or 2100 Bioanalyzer (Agilent Technologies Inc.) that allow assessing the quality of FFPE RNA samples based on DV200 values, which provides a reliable classification of degraded RNA by size. Refer to Appendix C, p.15.

On UV-spectrophotometer, RNA extracted from FFPE tissue commonly shows lower A260/A280 and/or A260/A230 values than recommended for standard RNA samples (A260/A280 ratio between 1.8 and 2.1. The A260/A230 ratio should be greater than 1.8). Always use provided low-TE buffer as a blank.

RNA Yield

We highly recommend using benchtop fluorometric assays, e.g., Qubit, to assess the RNA concentration. Please note that One-step purification technology results in higher final volume than most conventional silica-based methods and consequently lower concentration, yet without affecting the total RNA yield. RNA yield highly depends on the used input material, typically ranging from 100 ng - 6 µg per 1x10 µm tissue section.

Genomic DNA Contamination

The SPLIT One-step FFPE RNA Extraction Kit includes a DNase digestion step that effectively eliminates genomic DNA. The purified RNA is suitable for RNA-Seq or other downstream applications. However, certain samples, such as tissues with high blood content, may contain elevated DNA levels. In such cases, additional DNase (Cat. No. 235) treatment of the eluate and subsequent purification might be necessary.

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 using a fluorometric assay with DNA- and RNA-specific dyes to check samples for DNA contamination. On a denaturing 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. Alternatively, genomic DNA presence can be detected using qRT-PCR by analyzing a control sample without reverse transcription. If RNA is free of gDNA, no amplification product is observed.

8. Appendix C: Typical Results

The quality of the purified RNA is greatly influenced by the quality of the input material and the type of tissue.

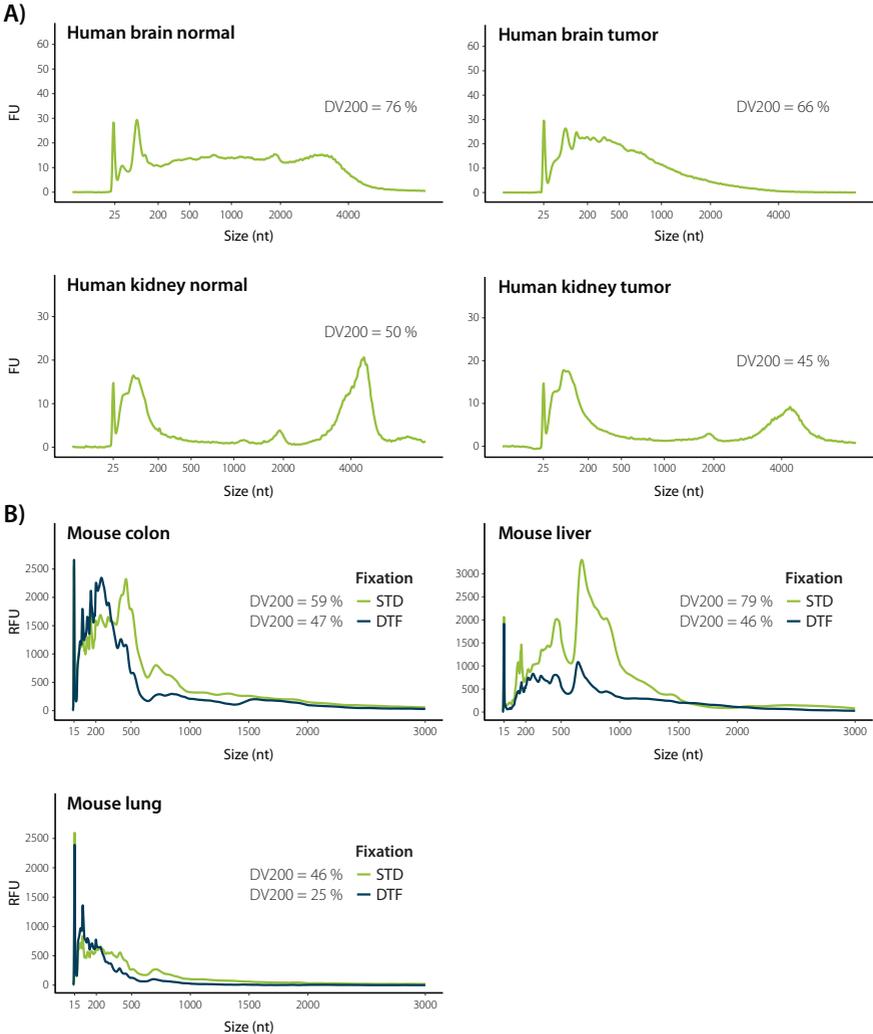


Figure 2. A) The 2100 Bioanalyzer traces (RNA 6000 pico assay) show examples of different human tissue types, with DV200 values ranging from 45% to 76%. B) The fragment Analyzer traces (High Sensitivity RNA Assay) of matching FFPE murine samples that were prepared with either standard fixation protocol (STD) or the fixation was deliberately delayed - tissue was kept on ice for 2 h before fixation (DTF). Consequently, the DV200 values of fixation-delayed samples are lower due to increased RNA degradation before embedding.

9. Appendix D: Revision History

Publication No. / Revision Date	Change	Page
236UG788V0100 May 6, 2024	Initial Release.	

Associated Products:

- 219-220 (CORALL FFPE Whole Transcriptome RNA-Seq Library Prep Kit with UDI 12 nt Set A1 or B1)
- 233-234 (RiboCop (HMV V2) and CORALL FFPE RNA-Seq Library Prep Kit with UDI 12 nt Set A1 or B1)
- 222 (QuantSeq FFPE 3' mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set A1, or A1-A4)
- 223 (QuantSeq FFPE 3' mRNA-Seq Library Prep Kit FWD with UDI 12 nt Set B1)
- 235 (DNA Removal Add-on)
- 239 (Column adapters for a plate centrifuge)

SPLIT One-step FFPE RNA Extraction Kit · User Guide

Lexogen GmbH
Campus Vienna Biocenter 5
1030 Vienna, Austria
Telephone: +43 (0) 1 345 1212-41
Fax: +43 (0) 1 345 1212-99
E-mail: support@lexogen.com
© Lexogen GmbH, 2024

Lexogen, Inc.
51 Autumn Pond Park
Greenland, NH 03840, USA
Telephone: +1-603-431-4300
Fax: +1-603-431-4333
www.lexogen.com