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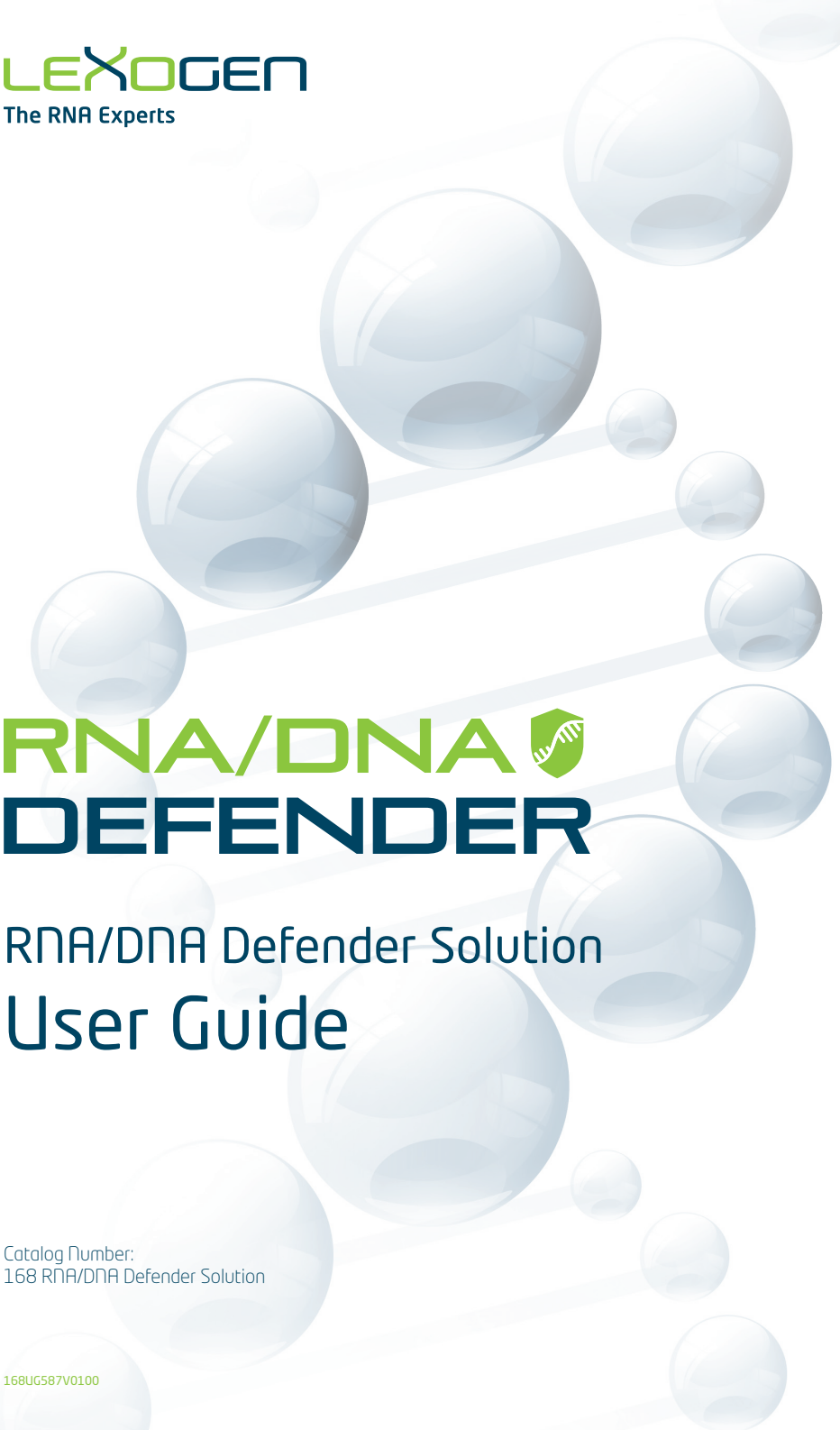
The RNA Experts



RNA/DNA DEFENDER

RNA/DNA Defender Solution

User Guide

Catalog Number:
168 RNA/DNA Defender Solution

168UG587V0100

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When describing a procedure for publication using this product, please refer to it as Lexogen's RNA/DNA Defender Solution (Cat. No. 168).

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1. Overview

Lexogen's RNA/DNA Defender Solution is a storage reagent that allows the storage of fresh tissue or cell samples after harvest without compromising the integrity of contained nucleic acids. Once deposited in RNA/DNA Defender Solution, samples can be stored for one day at 37 °C, one week at room temperature, one month at 4 °C, or up to one year at -20 °C.

The RNA/DNA Defender Solution rapidly permeates tissues and cells and stabilizes contained RNA and DNA molecules. Preserving RNA molecules in samples is crucial for maintaining the gene expression profile. RNA/DNA Defender Solution enables temporary storage of the specimen at temperatures where normally RNA degradation would occur, eliminating the need to snap-freeze the samples or to process them immediately.

For RNA extraction after storage, the tissue has to be removed from the solution and processed as instructed by a method of choice. We recommend Lexogen's SPLIT RNA Extraction Kit for the extraction of high-quality RNA for demanding downstream applications such as Next Generation Sequencing. The SPLIT RNA Extraction User Guide lists different extraction protocols for different sample types.

RNA/DNA Defender Solution can be used for most animal and plant tissues and cultured cells. However, depending on the specimen, downstream RNA extraction protocols will differ and need to be adjusted by the user.

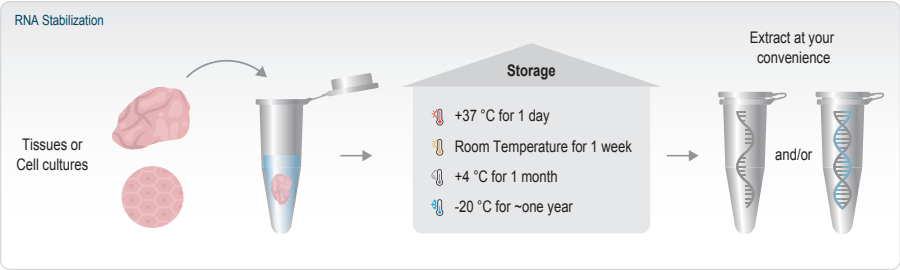

Figure 1. Workflow for storage of fresh tissue or cells in RNA/DNA Defender Solution for downstream RNA or DNA extraction.

2. Kit Components and Storage Conditions

Kit Component	Volume	Storage
RNA/DNA Defender Solution	100 ml	RT

Upon receiving the **RNA/DNA Defender Solution**, store bottle at room temperature.

NOTE: Check the **RNA/DNA Defender Solution** before use, as it may precipitate during shipping and storage. If a precipitate is visible, incubate at 37 °C with agitation until the precipitate dissolves completely.

3. User-Supplied Consumables and Equipment

Ensure that you have all of the necessary material and equipment before beginning the procedure. 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.

Reagents / Solutions

- RNA extraction method or kit of choice. We recommend Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008).

Equipment

- Calibrated single-channel pipettes for handling 10 µl to 1,000 µl volumes.
- Precision balance.

Labware

- Sterile scalpel, tweezers, and gauze pad.
- Suitable pipette tips (pipette tips with aerosol barriers recommended).
- 1.5 ml and 2.0 ml tubes with cap, low binding, certified ribonuclease-free.

Optional Equipment and Reagents

- Bench-top centrifuge (3,000 x g, rotor compatible with 1.5 ml tubes) for harvesting cell culture samples.
- Thermocycler.
- Bench-top cooler or ice pellets in ice box (for short-term storage of RNA).
- Vortex mixer.
- Phosphate Buffered Saline (1x PBS).

The complete set of material, reagents, and labware necessary for RNA extraction and quality control is not listed.

4. Detailed Protocol

4.1. Sample Preparation for Storage

ATTENTION: All steps, from harvesting tissue or cells to submerging in **RNA/DNA Defender Solution**, should be carried out as quickly as possible to avoid RNA degradation.

4.1.1. Animal and Plant Tissue

ATTENTION: Do not freeze tissues before submerging in **RNA/DNA Defender Solution**.

NOTE: Some plant tissues, e.g., waxy-coated leaves, will require physical disruption to allow the **RNA/DNA Defender Solution** to permeate into them completely. In this case, the protocol has to be adjusted.

1	Extract desired tissue.
2	Use sterile tweezers to transfer a tissue piece onto a fresh, sterile gauze pad.
3	Cut large tissue samples into smaller pieces of ≤5 mm in at least one dimension.
4	Determine the weight of the tissue piece on a precision balance.
5	Transfer the tissue sample into a suitable fresh tube.
6	Add ≥5 volumes of **RNA/DNA Defender Solution** to the sample, such that the tissue is completely submerged to allow full permeation. **EXAMPLE:** 50 mg tissue require ≥250 µl of **RNA/DNA Defender Solution**.
7	Store the submerged tissue at +4 °C to 37 °C. For details see chapter 4.2.

4.1.2. Cultured Cells

1	Harvest and pellet approximately 1 million cells according to your laboratory workflow for the respective cell line.
2	Remove and discard the supernatant. Do not disturb the pellet.
3	**OPTIONAL:** Carefully resuspend cell pellet in 100 µl 1x PBS.
4	Add ≥5 volumes (if cells were resuspended in 1x PBS use ≥500 µl) of **RNA/DNA Defender Solution** to the cells. Mix by inverting.
5	Transfer the tissue sample into a suitable fresh tube.
6	Store the resuspended cells at +4 °C to 37 °C. For details see chapter 4.2.

4.2. Sample Storage in RNA/DNA Defender Solution

Tissues submerged in RNA/DNA Defender Solution and cells resuspended in RNA/DNA Defender Solution can be stored for one day at 37 °C, one week at room temperature, one month at +4 °C, or up to one year at -20 °C.

4.3. RNA Extraction From Samples in RNA/DNA Defender Solution

To extract high-quality RNA from samples stored in RNA/DNA Defender Solution, we recommend Lexogen's SPLIT RNA Extraction Kit (Cat. No. 008). Refer to the respective User Guide for detailed instructions for the different sample types.

Tissue pieces submerged in RNA/DNA Defender Solution are removed from the solution, dried on a fresh and sterile gauze pad, and the weight is determined for the addition of the optimal volume of Isolation Buffer (**IB**). Then homogenization is carried out on the tissue following the SPLIT RNA Extraction User Guide.

Cells resuspended in RNA/DNA Defender Solution are pelleted, and the supernatant is removed before adding Isolation Buffer (**IB**). **NOTE:** If cell pelleting is unsuccessful, the sample can be diluted with one volume of 1x PBS and pelleting repeated.

The yield and RNA quality will depend on the efficiency of disruption and homogenization. The above steps may have to be adjusted to be compatible with downstream RNA extraction workflow.

Storage of tissue and cells in RNA/DNA Defender Solution is compatible with most RNA extraction methods, such as acid phenol extractions or column-based one-step kits.

4.4. DNA Extraction From Samples in RNA/DNA Defender Solution

Samples stored in RNA/DNA Defender Solution can also be used for extraction of DNA using any method of choice. Quality and yield of the extracted DNA depend on the chosen procedure and handling.

5. Appendix A: Revision History

Publication No. / Revision Date	Change	Page
168UG587V0100 May 23, 2023	Initial Release.	

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Associated Product:

008 (SPLIT RNA Extraction Kit)

RNA/DNA Defender Solution · User Guide

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