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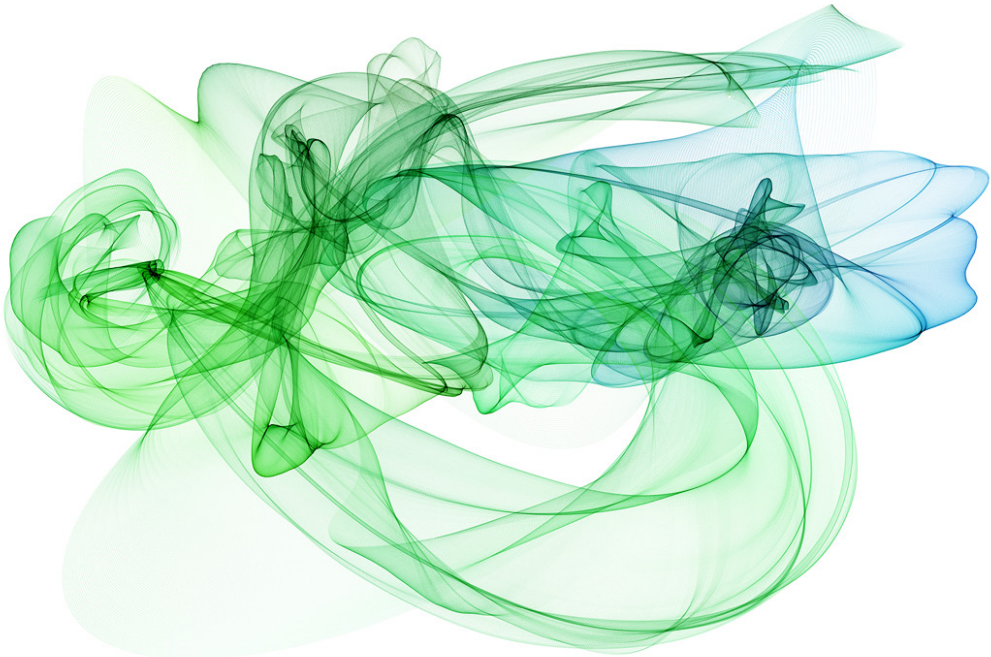
RNAstorm™

Fresh Cell and Tissue Kit

Kit Manual

50 extractions (CD504)



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RNAstorm™ Kit for Isolation of RNA from Fresh Cells and Tissues

About the Kit

Various methods in molecular biology, including next-generation sequencing (RNA-Seq), RT-PCR, or microarrays rely on RNA as input. The success of these methods directly depends on the ability to reliably extract high quality RNA from a variety of cells and tissues. The RNAstorm™ kit provides a convenient and efficient method of isolating RNA from cell cultures and fresh/frozen tissues.

⚠ Safety

Use of this product in a manner inconsistent or not specified in the provided instructions may result in personal injury or damage to equipment. Prior to use, ensure that all users of this product have received training in and are familiar with both general laboratory safety practices as well as the specific safety information associated with this product.

Safety information, including safety data sheets, can be accessed at http://celldatasci.com/safety.

Overview

Protocol Outline

A typical RNA isolation requires approximately 20-30 minutes and involves the following steps:

- Lysis: the sample is treated with Buffer FRL to lyse the sample and release RNA. Any RNases present in the sample are denatured and inactivated at this step.
- Binding to Spin Column: in the presence of Buffer FRB, the lysate is applied to a spin column, immobilizing the nucleic acids in the sample.
- DNase I Treatment (Optional): contaminating genomic DNA is degraded using DNase I. This step is optional but highly recommended.
- Washing and Elution of RNA: the column is washed twice using Wash Buffer. Pure RNA is finally eluted using water.

Kit Contents

The following reagents are included with the RNAstorm™ kit:

Reagent	Volume	Notes
Buffer FRL	36 mL	
Buffer FRB	15 mL	
DNase Buffer	5 mL	
Wash Buffer	12 mL	Prior to use, add 48 mL ethanol.
DNase I	Dried	Prior to use, reconstitute in 120 µL H_2O.
Spin Columns	50 ea	

Storage Conditions:

- All components of the kit should be stored at room temperature.
- After reconstitution, DNase I solution should be stored at -20˚C.

Before You Start

Please ensure that you have the following supplies and equipment ready, which are not provided with the kit:

- Beta-mercaptoethanol or DTT (optional; useful for tissues and cells containing high levels of RNases)
- Ethanol (200 proof, molecular biology grade).
- 1.5 mL microcentrifuge tubes (Eppendorf® DNA/RNA LoBind recommended).
- Microcentrifuge (12,000 rcf minimum).
- RNase-free water for DNase I reconstitution and final RNA elution step.
- Wash Buffer: ensure that 48 mL (for the 50 reaction kit) of 200 proof ethanol has been added to the provided bottle.
- DNase I: Reconstitute the lyophilized DNase I by adding 120 µL of RNase-free water. Using a pipette, mix gently to ensure the DNase is fully reconstituted. Briefly spin down tube if needed. To avoid repeated freezing and thawing of DNase, it is helpful to make aliquots as needed. Store the aliquots at -20°C.

Preparing the Cells or Tissue

The RNAstorm™ kit can be used with either animal cells or tissues (fresh or fresh/frozen).

If using cells:

To a maximum of 10^7 cells add an appropriate volume of Buffer FRL (see Table below) to a cell pellet. Mix by pipetting or vortexing. Proceed to step 1. Note: Cell culture medium may inhibit lysis. Before starting ensure cell culture medium has been thoroughly removed.

If using animal tissues:

To a maximum of 30 mg of tissue add an appropriate volume of Buffer FRL (see Table below). Homogenize tissue using either a tissue disruptor/homogenizer, mortar and pestle, or needle and syringe.

Sample	Sample quantity	Amount of Buffer FRL
Cells	$< 5 \times 10^6$	350 µL
	$\leq 1 \times 10^7$	600 µL
Tissues	< 20 mg	350 µL
	≤ 30 mg	600 µL

Detailed RNA Isolation Protocol

Lysis

1. Centrifuge the lysate for 3 min at 16,000 rcf. Carefully transfer the supernatant to a clean 1.5 mL Eppendorf tube.
2. Add an equivalent volume of 70% ethanol to the lysate. Mix well by pipetting. Do not centrifuge. Immediately proceed to the following step.
3. Transfer up to 700 µL of the sample, including any precipitate, to a spin column. Centrifuge for 30 seconds at 16,000 rcf. Discard the flow-through.
4. Repeat Step 3 until the entire sample has been passed through the spin column.

DNase I Treatment (Optional but Recommended)

This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 9.

5. Mix 240 µL of Buffer FRB and 360 µL of ethanol in a separate tube, for a total volume of 600 µL.
6. Add 300 µL of this mixture to the column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.
7. Mix 70 µL DNase I Buffer with 2 µL of reconstituted DNase I, and add this mixture directly to the center of the membrane of the spin column. Let stand for 15 minutes at room temperature.
8. Add the remaining 300 µL of the Buffer FRB/ethanol mixture (prepared in Step 5) to the column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.

Continue RNA Isolation

9. Add 500 µL of Wash Buffer to the spin column. Close the lid, and centrifuge for 30 seconds at 16,000 rcf. Discard the flow-through.
10. Wash again by repeating Step 9.
11. Dry the spin column by placing it back into an emptied collection tube and spinning again for 3 minutes at 16,000 rcf. Discard flow-through and collection tube. Place the spin column in a clean 1.5mL tube.
12. Elute the RNA by adding 50 µL of nuclease-free water to the center of the membrane of the spin column. Let stand for 1 minute. Centrifuge for 1 minute at 16,000 rcf. Note: RNA can be eluted in volumes as low as 30 µL, but total yield may be less.
13. Eluted RNA should be stored at -80˚C.

Terms and Conditions

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