

Product Information

RNAstorm™ RNA Isolation Kit

Catalog Number: CD504

Kit Size: 50 preps

Kit Components:

Component	Amount
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 uL H ₂ O)
Spin Columns	50 columns

Storage and Handling

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

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.

Product Description

The RNAstorm™ RNA Isolation Kit provides high quality total RNA from cultured cells or fresh tissue in as little as 20 minutes. The kit is based on a column purification method, and thus avoids toxic chemicals such as phenol or chloroform. RNA yields of up to 120 µg can be obtained, however this will depend on tissue type and condition. Contaminating DNA is removed using a DNase treatment step. The RNAstorm™ RNA Isolation Kit provides reliable extraction of high quality RNA which is required for many molecular biology applications, such as next-generation sequencing (RNA-Seq), RT-PCR, cDNA synthesis, and microarrays.

Product Protocol

Materials Required But Not Supplied:

- Beta-mercaptoethanol or DTT (optional; useful for tissues and cells containing high levels of RNases).
- Ethanol (200 proof, molecular biology grade).
- RNase-free water for DNase I reconstitution and final RNA elution step.

Before you Begin

Prepare the following buffer

Wash Buffer: ensure that 48 mL (for the 50 reaction kit) of 200 proof ethanol has been added to the provided bottle.

Prepare the DNase I

Reconstitute the lyophilized DNase I by adding 120 uL of RNase-free water. Using a pipette, gently mix 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.

Sample Lysis

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

If using cells:

Using the table below, add an appropriate volume of Buffer FRL to a cell pellet containing $\leq 10^7$ cells. 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:

Using the table below, add an appropriate volume of Buffer FRL to ≤ 30 mg of tissue. 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 uL
	$\leq 1 \times 10^7$	600 uL
Tissues	< 20 mg	350 uL
	≤ 30 mg	600 uL

RNA Isolation

1. Centrifuge the lysate for 3 minutes 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 uL 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 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 uL of Buffer FRB and 360 uL of ethanol in a separate tube, for a total volume of 600 uL.
6. Add 300 uL of this mixture to the spin column. Centrifuge for 30 seconds at 16,000 rcf and discard the flow-through.
7. Mix 70 uL DNase I Buffer with 2 uL 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 uL 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 uL 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 Eppendorf tube.
12. Elute the RNA by adding 50 uL 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 uL, but total yield may be less.
13. Eluted RNA should be stored at -80°C.

Related Products

Cat. No.	Product Name
41024	Water, Ultrapure Molecular Biology Grade
22020	10X Phosphate-Buffered Saline (PBS)
CD202	DNASTorm™ Kit for Isolation of DNA from FFPE Tissue Samples
CD201	RNAStorm™ Kit for Isolation of RNA from FFPE Tissue Samples
31030	DNA Gel Extraction Kit
31007	AccuBlue® Broad Range dsDNA Quantitation Kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
31060	AccuBlue® NextGen dsDNA Quantitation Kit
31066	AccuGreen™ High Sensitivity dsDNA Quantitation Kit (for Qubit®)
41003	GelRed® Nucleic Acid Gel Stain, 10,000X in Water
41005	GelGreen® Nucleic Acid Gel Stain, 10,000X in Water
41042	DNAzure® Blue Nucleic Acid Gel Stain
E90003	Gel-Bright™ LED Gel Illuminator
31022	Ready-to-Use 1 kb DNA Ladder
31032	Ready-to-Use 100 bp DNA Ladder
31042	Forget-Me-Not™ EvaGreen® qPCR Master Mix
31043	Forget-Me-Not™ Universal Probe Master Mix
31000	EvaGreen® Dye, 20X in Water

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