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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit

Catalog Number: CD504 (50 preps)

Kit Contents

Component	Size
99872: RNAstorm™ Fresh Lysis Buffer	36 mL
99873: RNAstorm™ Fresh Binding Buffer	15 mL
99865: DNase Buffer	5 mL
99867-3000U: DNase I (lyophilized)	3000 U (Reconstitute in 120 uL water prior to use)
99864: CELLDATA Wash Buffer	12 mL (Add 48 mL ethanol prior to use)
99868-50: CELLDATA Spin Columns	50 each

Storage and Handling

Upon receipt, store DNase I at 2-8°C. After reconstitution, DNase I solution should be stored at -20°C. Store other kit components at room temperature. Kit components are stable for at least 9 months from date of receipt when stored as recommended. The Lysis Buffer and Binding Buffer contain chaotropic guanidine salts, which are hazardous (see SDS). Use gloves and other appropriate laboratory protection when using this kit. Mixing bleach with guanidine salts can produce hazardous byproducts. DO NOT mix waste from this kit with bleach. Handle all kit components using universal laboratory safety precautions.

Product Description

The CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit provides high quality total RNA from cultured cells or fresh tissue for use with various downstream applications, including next-generation sequencing (RNA-Seq), RT-PCR, cDNA synthesis, and microarrays. The RNA isolation procedure is a simple column purification method that takes as little as 20 minutes and requires no phenol/chloroform or ethanol precipitation steps. The RNA recovery will depend primarily on the condition and type of cells or tissue, but with samples of good quality it is possible to recover up to 120 ug of total RNA per sample. An optional but strongly recommended DNase treatment step is used to remove contaminating DNA.

Product Protocol

Materials required but not supplied

- Beta-mercaptoethanol (β-ME) or dithiothreitol (DTT) (optional; useful for tissues and cells containing high levels of RNases)
- Ethanol (200 proof, molecular biology grade); dilute to 70% ethanol with RNase-free water for RNA isolation step (see Related Products for Water, Ultrapure Molecular Biology Grade)
- 1.5 mL microcentrifuge tubes (Eppendorf LoBind® tubes recommended)
- Microcentrifuge (12,000 x g minimum)
- RNase-free water for DNase I reconstitution and final RNA elution step (see Related Products for Water, Ultrapure Molecular Biology Grade)

Before you begin

Prepare the following buffers

- CELLDATA Wash Buffer: Add 48 mL of 200 proof ethanol to the bottle and mix well. Mark the ethanol added box on the label.
- DNase I: Reconstitute the lyophilized DNase I by adding 120 uL of RNase-free water. Using a pipette, mix gently to ensure the DNase is fully reconstituted (do not vortex, which can denature DNase). Briefly centrifuge the tube to collect contents at bottom. Store in aliquots at -20°C and avoid freeze/thaw cycles.

Sample lysis

The CELLDATA RNAstorm™ Fresh Cell and Tissue RNA Isolation Kit can be used with either animal cells or tissues (fresh or fresh/frozen). If purifying RNA from cells or tissues rich in RNases, we recommend adding β-ME to the RNAstorm™ Fresh Lysis Buffer before use. Add 1 uL β-ME per 100 uL of aliquoted RNAstorm™ Fresh Lysis Buffer in a fume hood and wear appropriate protective clothing. Alternatively, add 2 uL of 2 M DTT per 100 uL of aliquoted RNAstorm™ Fresh Lysis Buffer. The stock solution of 2 M DTT in water should be prepared fresh or stored as frozen single-use aliquots. RNAstorm™ Fresh Lysis Buffer containing β-ME or DTT can be stored at room temperature for up to 1 month.

If using cells:

Using Table 1 below, add an appropriate volume of RNAstorm™ Fresh Lysis Buffer to the cell pellet.

Note: Cell culture medium may inhibit lysis. Before starting, ensure cell culture medium has been thoroughly removed.

If using animal tissues:

Using Table 1 below, add an appropriate volume of RNAstorm™ Fresh Lysis Buffer to the tissue. Homogenize tissue using either a tissue disruptor/homogenizer, mortar and pestle, or needle and syringe.

Table 1. Lysis Buffer Volume Required

Sample	Sample Quantity	Amount of RNAstorm™ Fresh Lysis Buffer
Cells	< 5 x 10^6	350 uL
	0.5 - 1 x 10^7	600 uL
Tissues	< 20 mg	350 uL
	20 - 30 mg	600 uL

1. RNA isolation

1.1 Centrifuge the lysate for 3 minutes at 16,000 x g. Carefully transfer the supernatant to a clean 1.5 mL microcentrifuge tube.

1.2 Add an equivalent volume of 70% ethanol to the supernatant. Mix well by pipetting. Do not centrifuge. Immediately proceed to the following step.

1.3 Transfer up to 700 uL of the sample, including any precipitate, to a spin column. Centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

1.4 Repeat Step 1.3 until the entire sample volume has passed through the spin column.

2. DNase I treatment (recommended)

Note: This step ensures that any contaminating genomic DNA is degraded. To skip DNase I treatment, proceed to step 3.1.

2.1 Mix 240 uL of RNAstorm™ Fresh Binding Buffer and 360 uL of 100% ethanol (200 proof) in a separate tube, for a total volume of 600 uL.

2.2 Add 300 uL of this mixture to the spin column. Centrifuge for 30 seconds at 16,000 x g and discard the flow-through.

2.3 Mix 70 uL of DNase 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.

2.4 Add the remaining 300 uL of the RNAstorm™ Fresh Binding Buffer/ethanol mixture (prepared in step 2.1) to the column. Centrifuge for 30 seconds at 16,000 x g and discard the flow-through.

3. Continue RNA isolation

3.1 Add 500 uL of CELLDATA Wash Buffer to the spin column. Close the tube lid, and centrifuge for 30 seconds at 16,000 x g. Discard the flow-through.

3.2 Wash again by repeating step 3.1.

3.3 Dry the spin column by placing it back into an emptied collection tube and centrifuging again for 3 minutes at 16,000 x g. Discard the flow-through. Place the spin column in a clean microcentrifuge tube.

3.4 Elute the pure 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 x g. Collect the eluted RNA from the centrifuge tube.

Note: RNA can be eluted in volumes as low as 30 uL, but total yield may be less.

3.5 Optional: Repeat step 3.4 for a higher RNA yield but at a lower concentration.

3.6 Eluted RNA should be stored at -80°C.

Related Products

Catalog number	Product
41024-4L	Water, Ultrapure Molecular Biology Grade
CD502	CELLDATA DNAstorm™ FFPE DNA Extraction Kit
CD501	CELLDATA RNAstorm™ FFPE RNA Extraction Kit
41032	EMBER500™ RNA Prestain Loading Dye
31073	AccuBlue® Broad Range RNA Quantitation Kit
31028	AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit
22028	RNase-X™ Decontamination Solution
31030	DNA Gel Extraction Kit
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
31022	Ready-to-Use 1 kb DNA ladder
31032	Ready-to-Use 100 bp DNA ladder
31042	Forget-Me-Not™ qPCR Master Mix
31043	Forget-Me-Not™ Universal Probe Master Mix
31077	EvaGreen® Plus Dye, 20X in water
31000	EvaGreen® Dye, 20X in water

Please visit our website at www.biotium.com for information on our products for RNA research and applications, including RNA extraction kits for fresh cells and FFPE tissues, RNA quantitation kits, and RNA gel stains.