Biotium
Glowing Products for Science™

interchim

www.biotium.com

January 7, 2019

# 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<br>(Prior to use, add 48 mL ethanol) |
| DNase I | Dried<br>(Prior to use, reconstitute in 120 uL $H_2O$) |
| 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 ≤ 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. # | Product |
|---|---|
| 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 |

Please visit our website at www.biotium.com for information on our life science research products, including qPCR master mixes, fluorescent CF® dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.