TECHNICAL MANUAL

# ReliaPrep™ RNA Clean-Up and Concentration System

Instructions for Use of Products
**Z1071, Z1072 and Z1073**


6/18
TM541

# ReliaPrep™ RNA Clean-Up and Concentration System

**All technical literature is available at: www.promega.com/protocols/**
**Visit the web site to verify that you are using the most current version of this Technical Manual.**
**E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com**



## 1. Description

The purity and concentration of RNA prepared from conventional isolation protocols (organic extraction or column-based procedures) is often affected by carryover of purification reagents like guanidine salts and ethanol. Furthermore, the requirements of some purification kits for large elution volumes can leave the user with RNA too dilute for some downstream applications. Additionally, a simpler and faster protocol for enzymatic RNA reactions (reverse transcription or post-DNase treatment) is also desired. The ReliaPrep™ RNA Clean-Up and Concentration System was designed to address these needs while maintaining good recovery of RNA samples and ensuring their usefulness in downstream applications such as RT-qPCR, Northern blot analysis or RNA sequencing. An added benefit is that dilute samples or sample pools up to 300µl can be processed over a single column and eluted in 15µl of water or TE buffer. You can process up to 12 samples in less than 10 minutes.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT # |
|---|---|---|
| **ReliaPrep™ RNA Clean-Up and Concentration System** | **250 preps** | **Z1073** |

For Research Use. Each system contains sufficient reagents for 250 RNA purifications. Includes:

- 5 packs Reliaprep™ Minicolumns (50/pack)
- 1 pack Collection Tubes (250/pack)
- 5 packs Elution Tubes (50/pack)
- 2 × 20ml Membrane Binding Solution
- 24ml Column Wash Solution (CWE)
- 58.8ml RNA Wash Solution (RWA)
- 25ml Nuclease-Free Water

| PRODUCT | SIZE | CAT # |
|---|---|---|
| **ReliaPrep™ RNA Clean-Up and Concentration System** | **50 preps** | **Z1072** |

For Research Use. Each system contains sufficient reagents for 50 RNA purifications. Includes:

- 1 pack Reliaprep™ Minicolumns (50/pack)
- 1 pack Collection Tubes (50/pack)
- 2 packs Elution Tubes (25/pack)
- 20ml Membrane Binding Solution
- 5ml Column Wash Solution (CWE)
- 11.8ml RNA Wash Solution (RWA)
- 1.25ml Nuclease-Free Water

| PRODUCT | SIZE | CAT # |
|---|---|---|
| **ReliaPrep™ RNA Clean-Up and Concentration System** | **10 preps** | **Z1071** |

For Research Use. Each system contains sufficient reagents for 10 RNA purifications. Includes:

- 1 pack Reliaprep™ Minicolumns (10/pack)
- 1 pack Collection Tubes (10/pack)
- 2 packs Elution Tubes (5/pack)
- 4ml Membrane Binding Solution
- 1ml Column Wash Solution (CWE)
- 11.8ml RNA Wash Solution (RWA)
- 1.25ml Nuclease-Free Water

**Storage Conditions:** All components can be stored at 15–30°C.

## 3. General Considerations

### 3.A. Processing Capacity

Each column is capable of binding as much as 80µg of RNA. However, if concentrating or cleaning up RNA preparations of greater than 10µg, we recommend eluting twice or increasing elution volume.

### 3.B. Downstream Applications

RNA purified using the ReliaPrep™ RNA Clean-Up and Concentration System is suitable for use in many molecular biology applications such as RT-qPCR, Northern blotting or next-generation sequencing.

For all downstream applications, continue to protect your samples from RNases by wearing gloves and using solutions and tubes that are RNase-free. Using an RNase inhibitor such as Recombinant RNasin® Ribonuclease Inhibitor (Cat.# N2511) can ensure protection from nucleases that might be introduced during downstream processing.

### 3.C. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Avoid inadvertently introducing RNases into your RNA during or after the purification. This is especially important if the RNA sample was difficult to obtain or is irreplaceable. The following notes may help you to prevent accidental RNase contamination of your sample:

1. Two of the most common sources of RNase contamination are the user's hands or bacteria and molds present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with the kit. Wear gloves at all times and use eye protection.
2. Whenever possible, sterile disposable plasticware should be used for handling RNA. These materials are generally RNase-free and thus do not require pretreatment to inactivate RNases. Sterile elution tubes are provided.
3. Reagents contain irritants and should be properly disposed of as hazardous waste. Do not use products containing bleach for sanitation.
4. Change pipette tips frequently to prevent sample cross-contamination.

## 4. RNA Purification and/or Concentration

For best results, do not stop the procedure once samples are loaded onto the column. After elution, store samples at –70°C. If desired, RNA can be eluted in TE buffer (not provided).

### Materials to be Supplied by the User

- 100% isopropanol, RNase-free
- 95–100% ethanol, RNase-free
- 1.5ml microcentrifuge tubes
- microcentrifuge capable of maintaining 10,000 × *g* (14,000rpm)

### 4.A. Preparing Solutions

| Solution | Preparation | Notes |
|---|---|---|
| Column Wash Solution (CWE) | **250 prep size** (Part# A254C): Add 36ml of 95–100% ethanol to the bottle containing 24ml of concentrate. | After adding ethanol, mark on the bottle label that this step has been performed. The reagent is stable at 15–30°C when tightly capped. |
| | **50 prep size** (Cat.# A254B): Add 7.5ml of 95–100% ethanol to the bottle containing 5ml of concentrate. | |
| | **10 prep size** (Part# A254A): Add 1.5ml of 95–100% ethanol to the bottle containing 1ml of concentrate. | |
| RNA Wash Solution (RWA) | **250 prep size** (Part# Z3091): Add 100ml of 95–100% ethanol to the bottle containing 58.8ml of concentrate. | After adding ethanol, mark on the bottle label that this step has been performed. The reagent is stable at 15–30°C when tightly capped. |
| | **50 prep size** (Part# Z309B): Add 20ml of 95–100% ethanol to the bottle containing 11.8ml of concentrate. | |
| | **10 prep size** (Part# Z309B): Add 20ml of 95–100% ethanol to the bottle containing 11.8ml of concentrate. | |
| Membrane Binding Solution | Check that no precipitate is present in the bottle. | If there is precipitate, warm the contents in a 37°C waterbath, swirling until the precipitate redissolves. |

## 4.B. Purifying and/or Concentrating RNA Samples

**Notes:**

1. This protocol requires 100% isopropanol (not provided).
2. Perform all centrifugation steps at 10,000 × *g* (14,000rpm).

| Dilute RNA (µl) | Membrane Binding Solution (µl) | 100% Isopropanol (µl) | Total Load (µl) |
|---|---|---|---|
| 25 | 12.5 | 37.5 | 75 |
| 50 | 25 | 75 | 150 |
| 100 | 50 | 150 | 300 |
| 150 | 75 | 225 | 450 |
| 200 | 100 | 300 | 600 |
| 250 | 125 | 375 | 750 |
| 300 | 150 | 450 | 900 |

**Note:** You can process up to 300µl of dilute RNA at one time in a single minicolumn. If a dilute RNA sample exceeds 300µl, process the sample in multiple tubes in the manner described above and load the multiple centrifugations onto the same column. The wash protocol remains unchanged.

1. Pipet 25–300µl of dilute RNA into a 1.5ml microcentrifuge tube.
2. Add 1/2 volume of Membrane Binding Solution and vortex 5 seconds.
3. Add 1 1/2 volumes of 100% isopropanol.
4. Load RNA sample onto a ReliaPrep™ Minicolumn seated in a Collection Tube and centrifuge for 30 seconds.

   **Note:** This mixture is viscous so slowly transfer the sample to the column. Save the sample tubes after transfer to check that no significant volume remains.
5. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
6. Add 200µl of Column Wash Solution (CWE) and centrifuge for 15 seconds. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
7. Wash with 300µl of RNA Wash Solution (RWA) and centrifuge for 15 seconds. Repeat wash with 300µl of RNA Wash Solution (RWA) and centrifuge again.
8. Remove column and discard the contents of the collection tube. Reseat the column into the same collection tube, centrifuge for 1 minute to dry the column and then transfer column to an Elution Tube.
9. Pipet 15µl of Nuclease-Free Water or TE buffer (not provided) to the center of the column matrix and centrifuge for 30 seconds.

   **Note**: Touch the pipette tip to the column bed surface before dispensing Nuclease-Free Water or TE buffer to completely wet the column matrix. The color should change from light to dark tan.
10. For maximum recovery, repeat elution with an additional 15µl of Nuclease-Free Water or TE buffer.

## 5. Determining RNA Yield and Purity

The ReliaPrep™ RNA Clean-Up and Concentration System can be used to purify and concentrate RNA sourced from previous purification schemes or simply concentrate RNA preparations from dilute solutions. The total RNA yield may be determined spectrophotometrically at 260nM, where 1 absorbance unit ($A_{260}$) equals approximately 40µg of single-stranded RNA/ml. The purity also may be estimated by spectrophotometry from the relative absorbances at 230nm, 260nm and 280nm (i.e., $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios). If overall expected yield is less than 1µg, spectrophotometric analysis will not yield accurate results due to the lack of absorbance sensitivity for low-concentration nucleic acids. Alternatively, low RNA yield can be determined with good accuracy using the QuantiFluor® RNA System (Cat.# E3310).

RNA purified using the ReliaPrep™ RNA Clean-Up and Concentration System is substantially free of contaminating protein. Pure RNA should exhibit an $A_{260}/A_{280}$ ratio of 2.0. However, variations between individual starting materials and skill in performing the procedure may reduce this purity ratio to the 1.7–2.1 range. This is not necessarily problematic for downstream applications. RNA will usually exhibit an $A_{260}/A_{230}$ ratio of 1.8–2.2. A low $A_{260}/A_{230}$ ratio can indicate guanidine contamination, which may interfere with performance or accuracy of results in downstream applications. A low $A_{260}/A_{230}$ ratio may also indicate ethanol contamination. Be sure to follow the centrifugation recommendations in the protocol.

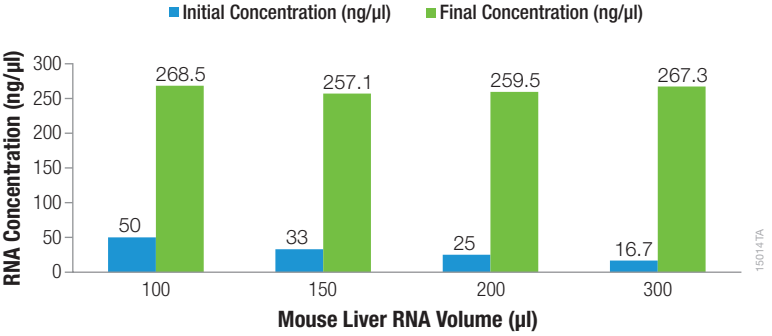


**Figure 1. Recovery and concentration of RNA from dilute solution using the ReliaPrep™ RNA Clean-Up and Concentration System.** Five micrograms of mouse liver RNA was suspended in 100, 150, 200 or 300µl of water, repurified using the standard protocol and eluted in 15µl of Nuclease-Free Water. Results shown in Figure 1 demonstrate equivalent yield regardless of target RNA concentration. Average RNA yield was 79.8%.

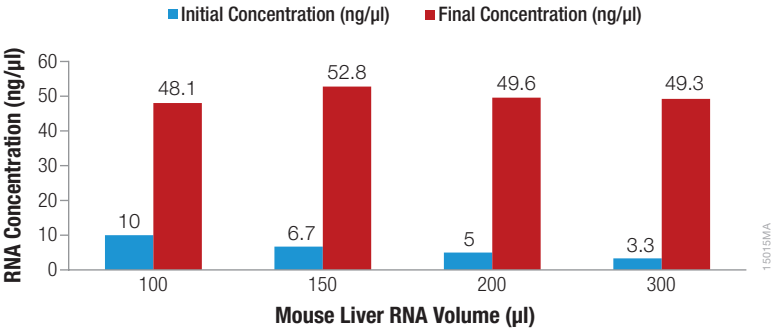


**Figure 2. Recovery and concentration of RNA from dilute solution using the ReliaPrep™ RNA Clean-Up and Concentration System.** One microgram of mouse liver RNA was suspended in 100, 150, 200 or 300µl of water, repurified using the standard protocol and eluted in 15µl of Nuclease-Free Water. Results shown in Figure 2 demonstrate equivalent yield regardless of target RNA concentration. Average RNA yield was 74.9%.

## 6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

| Symptoms | Possible Causes and Comments |
|---|---|
| Low $A_{260}/A_{280}$ ratios | Low RNA concentration. Spectrophotometric methods may not be accurate when expected RNA concentration drops below approximately 15ng/µl. As the signal approaches the limit of detection for the instrument, background noise may lead to inaccurate readings. |
| Low $A_{260}/A_{230}$ ratios | Spectrophotometric methods may not be accurate when expected RNA concentration drops below approximately 15ng/µl. As the signal approaches the limit of detection for the instrument, background noise may lead to inaccurate readings. |
| | Low $A_{260}/A_{230}$ ratios are typically due to guanidine thiocyanate contamination. Be certain to complete all wash steps in the protocol. |
| Low $A_{260}$ value | Low RNA yield. Spectrophotometric methods may not be accurate when expected RNA concentration drops below approximately 15ng/µl. As the signal approaches the limit of detection for the instrument, background noise may lead to inaccurate readings. |

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## 6. Troubleshooting (continued)

| Symptoms | Possible Causes and Comments |
|---|---|
| Low $A_{260}$ value (continued) | The correct reagent volume may not have been added during the protocol. The volumes used for the kit reagents are critical to the success of the procedure. |
| | Ethanol may not have been added to the Column Wash Solution or RNA Wash Solution. Prepare solutions as instructed in Section 4.A. |
| Calculated yield lower than expected | RNA may be retained on the minicolumn if expected yield is >10µg. Elute a second time with 15µl of Nuclease-Free Water. |
| RNA degradation | RNase was introduced during handling. Carefully handle kit reagent bottles to avoid contamination. Use the provided disposable plasticware when manipulating and storing RNA. Wear gloves at all times. |
| | RNA was degraded during sample preparation. Work quickly when preparing samples, and store eluates at –70°C. |

## 7. Related Products

| Product | Size | Cat.# |
|---|---|---|
| PureYield™ Plasmid Miniprep System | 100 preps | A1223 |
| | 250 preps | A1222 |
| PureYield™ Plasmid Midiprep System | 25 preps | A2492 |
| | 100 preps | A2495 |
| | 300 preps | A2496 |
| PureYield™ Plasmid Maxiprep System | 10 preps | A2392 |
| | 25 preps | A2393 |
| pGEM®-T Vector Systems | 20 reactions | A3600 |
| pGEM®-T Vector Systems + JM109 Competent Cells | 20 reactions | A3610 |
| pGEM®-T Easy Vector Systems | 20 reactions | A1360 |
| pGEM®-T Easy Vector Systems + JM109 Competent Cells | 20 reactions | A1380 |
| LigaFast™ Rapid DNA Ligation System | 30 reactions | M8221 |
| | 150 reactions | M8225 |
| QuantiFluor® RNA System | 1ml | E3310 |

**Note:** You can use the CloneWeaver™ Workflow Builder to design a cloning workflow for your construct: **www.promega.com/resources/tools/cloneweaver/**