



ZYMO RESEARCH

RNA
Purification
Made Simple

RNA Clean & Concentrator™ -25

Clean-up RNA from any sample

Highlights

- Quick, 5-minute clean-up of total RNA (including small/microRNAs) from any enzymatic reaction, aqueous phase following TRIZOL® extraction, in vitro transcription products, etc.
- Ultra-pure RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.

Catalog Numbers:
R1017, R1018



Scan with your smart-phone camera to
view the online protocol/video.



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Product Contents

RNA Clean & Concentrator™-25	R1017 (50 prep)	R1018 (100 prep)
RNA Binding Buffer	25 ml	50 ml
RNA Prep Buffer	25 ml	25 ml (x2)
RNA Wash Buffer (concentrate) ¹	12 ml	24 ml
DNase/RNase-Free Water	6 ml	10 ml
Zymo-Spin™ IICR Columns	50	100
Collection Tubes	50	100
Instruction Manual	1	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.

Before use:

1 Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1017) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1018).

Specifications

- **Sample Sources** – Enzymatic reactions (e.g., DNase I treated RNA), the aqueous phase following TRIzol®/chloroform or similar¹ extraction, in vitro transcriptions, etc.
- **Size** – Total RNA including small/microRNAs (≥ 17 nt).
- **Purity** – A_{260}/A_{280} & $A_{260}/A_{230} > 1.8$. RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.
- **Binding Capacity** – 50 μg total RNA (**Zymo-Spin™ IICR Column**).
- **Elution Volume** – ≥ 25 μl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) – Microcentrifuge.
- **Chemical Tolerance** – $\leq 5\%$ Triton X-100, $\leq 5\%$ Tween-20, $\leq 5\%$ Sarkosyl, $\leq 0.1\%$ SDS. Also compatible with $\leq 90\%$ Sucrose, $\leq 90\%$ Formamide, and $\leq 2\%$ Formaldehyde.

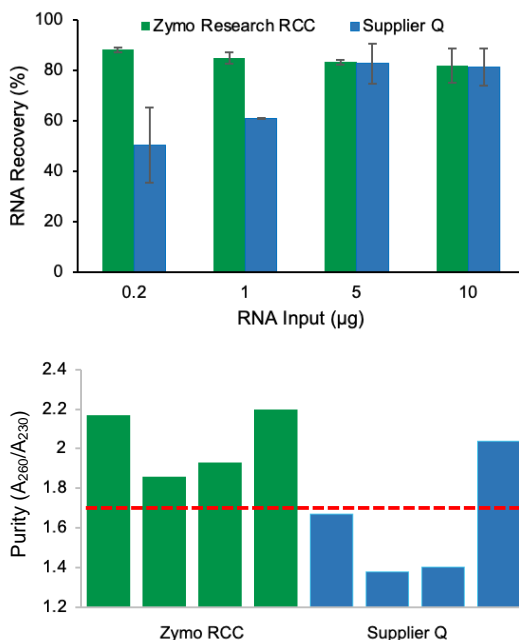
¹ TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.

Product Description

The **RNA Clean & Concentrator™-25** kit provides a simple and reliable method for the rapid preparation of up to 50 µg of high-quality, NGS-ready RNA. This 5 minute procedure is based on the use of a unique single-buffer system and Zymo-Spin™ technology that allows for selective recovery of total RNA (> 17nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

The procedure is easy: Add binding buffer and ethanol to your sample, then bind, wash and elute ultra-pure RNA. The RNA can be eluted from the **Zymo-Spin™ IICR Column** in as little as ≥ 25 µl of RNase-free water. The highly concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.

Consistent Recovery and Ultra-pure Total RNA



(top) Increasing amounts of RNA was cleaned up using the **RCC™** kit and a Supplier Q kit (n=2). **RCC™** provides higher yields and more consistent recovery when compared to the Supplier Q Kit. (bottom) RNA was cleaned using the **RCC™** kit and a Supplier Q kit (n=4). RNA purity (measured by A₂₆₀/230) was greater than 1.8 for the **RCC™** kit but not for the Supplier Q kit.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) Total RNA Clean-up.

(I) Buffer Preparation

- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1017) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1018).

(II) Total RNA Clean-up

- ✓ RNA species ≥ 17 nt will be recovered.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ For DNA-free RNA (optional), perform **DNase I** treatment before (recommended) or during clean-up (page 6).

1. Add 2 volumes **RNA Binding Buffer** to each sample¹ and mix.

Example: Mix 100 μ l buffer and 50 μ l sample.

2. Add an equal volume of ethanol² (95-100%) and mix.

Example: Add 150 μ l ethanol.

3. Transfer the sample to the **Zymo-Spin™ IICR Column**³ in a **Collection Tube** and centrifuge. Discard the flow-through.

Optional: At this point, in-column **DNase I** treatment can be performed (page 6).

4. Add 400 μ l **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 700 μ l **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 μ l **RNA Wash Buffer** to the column and centrifuge for 1 minute ensure complete removal of the wash buffer. Carefully, transfer the column into a RNase-free tube (not provided).

7. Add 50 μ l **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥ 25 μ l elution.

The eluted RNA can be used immediately or stored frozen.

1 To minimize pipetting error, adjust the sample volume to 50 μ l (minimum).

2 Alternatively, if working exclusively with RNA fragments 17-200 nt in length, use 1.5 volumes of 95-100% ethanol.

3 To process samples >700 μ l, **Zymo-Spin™** columns may be reloaded.

Appendices

DNase I Treatment

- ✓ For DNA-free RNA, **DNase I** treatment can be performed using **DNase I Set** (E1010; 50 reactions) and RNA Wash Buffer (concentrate) (R1003-3-6); materials sold separately.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

DNase I treatment before RNA clean-up (Recommended)

For each sample to be treated, prepare 50 µl **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion. Then incubate at room temperature (20-30°C) for 15 minutes and proceed with the RNA Clean-up protocol, page 5.

DNase I Reaction Mix

RNA sample (≤ 10 µg; volume adjusted with water or TE buffer)	40 µl
DNase I (reconstituted; 1 U/ul) ^{1,2}	5 µl
DNA Digestion Buffer	5 µl

In-column DNase I treatment

1. Following RNA binding step (page 5, step 3), add 400 µl **RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion. Then add 80 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the RNA Clean-up protocol (page 5, step 4).

DNase I Reaction Mix

DNase I (reconstituted; 1 U/ul) ^{1,2}	5 µl
DNA Digestion Buffer	75 µl

1 Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A260 units/ml of reaction mixture at 25°C.

2 Reconstitute lyophilized **DNase I** (#E1009-A; 250 U) with 275 µl **DNase/RNase-Free Water** and mix by gentle inversion. Store frozen aliquots.

Purification of Small and Large RNAs into Separate Fractions

- ✓ Perform all steps at room temperature and centrifugation steps at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ This protocol requires two columns per prep.

1. Prepare adjusted **RNA Binding Buffer** (as needed). Mix an equal volume of buffer and ethanol (95-100%).

Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the adjusted buffer to the sample¹ and mix.

Example: Mix 100 µl adjusted buffer and 50 µl sample.

3. Transfer the mixture to the **Zymo-Spin™ Column**² and centrifuge.
Save the flow-through!

4. **Small RNAs (17-200 nt)**
are in the flow-through

- a. Add 1 volume ethanol and mix.

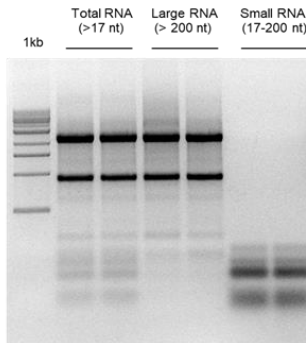
Example: Add 150 µl ethanol to 150 µl sample.

- b. Transfer the mixture to a **new column** and centrifuge. Discard the flow-through.

- c. Proceed with the RNA Clean-up protocol, page 5, step 4.

4. **Large RNAs (> 200 nt)**
are retained in the column

- a. Proceed with the RNA Clean-up protocol, page 5, step 4.



RNA Clean & Concentrator™ allows for clean-up of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).
2 To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

RNA clean-up from aqueous phase after TRIzol®/chloroform extraction

Following TRIzol®/chloroform or similar* extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided). Add 1 volume of ethanol (95-100%) to 1 volume of aqueous phase¹ (1:1) and mix well. Then proceed with the RNA Clean-up protocol, page 5, step 3.

RNA clean-up from samples in DNA/RNA Shield™

- ✓ Perform all steps at room temperature and centrifugation steps at 10,000-16,000 x g for 30 seconds, unless specified.
- 1. If frozen, thaw samples to room temperature (20-30°C) and centrifuge debris (if any). Transfer the cleared sample into an RNase-free tube (not provided).
- 2. Add 1 volume of ethanol (95-100%) to 1 volume of the **DNA/RNA Shield™** sample¹ and mix well.
Example: 50 µl buffer and 50 µl sample.
- 3. Continue with the RNA Clean-up protocol, page 5, step 3.

cDNA Clean-Up following Reverse Transcription (RT)

The **RNA Clean & Concentrator** can be used to effectively clean and concentrate first-strand cDNA following reverse transcription (RT) and hydrolysis. The **RNA Binding Buffer** will neutralize the hydrolysis reaction and the recovered cDNA may be used directly for microarray analysis, etc.

Hydrolysis Reaction: To each 30-50 µl RT reaction, add 10 µl 0.5 M EDTA and 10 µl 1 M NaOH. Then mix and incubate at 65°C for 15 minutes. Proceed to the Total RNA Clean-Up protocol, page 5.

* TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid guanidinium-phenol reagents.
1 To minimize pipetting error, adjust the sample volume to 50 µl (minimum).

Ordering Information

Product Description	Catalog No.	Size
RNA Clean & Concentrator™-25	R1017	50 preps.
	R1018	100 preps.

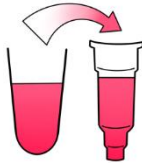
Individual Kit Components	Catalog No.	Amount
RNA Binding Buffer	R1013-2-25	25 ml
	R1013-2-50	50 ml
	R1013-2-100	100 ml
RNA Prep Buffer	R1060-2-25	25 ml
	R1060-2-100	100 ml
RNA Wash Buffer (concentrate)	R1003-3-24	24 ml
	R1003-3-48	48 ml
Zymo-Spin™ IICR Columns	C1078-50	50
	C1078-250	250
Collection Tubes	C1001-50	50
	C1001-500	500
DNase/RNase-Free Water	W1001-6	6 ml
	W1001-10	10 ml
DNase I Set (250 U DNase I (lyophilized) supplied with DNA Digestion Buffer, 4 ml)	E1010	1

Complete Your Workflow

- ✓ For tough-to-lyse samples in TRIzol, use ZR BashingBead Lysis Tubes:

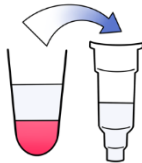
ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	For plant/animal tissue
0.1 + 0.5 mm beads #S6012	For microbes
0.1 + 2.0 mm beads #S6014	For microbes in tissue/insects

- ✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



Direct-zol RNA kits	
Microprep #R2060-R2063	From 1 cell and up
Miniprep #R2050-R2053	Up to 50 ug RNA
Miniprep Plus #R2070-R2073	Up to 100 ug RNA
96-well #R2054-R2057	Spin-plate
MagBeads #R2100-R2105	Automatable (Tecan, Hamilton, Kingfisher, etc.)

- ✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):






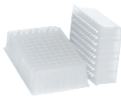
RNA Clean & Concentrator kit	
#R1013-R1014	DNase I Set included

- ✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Format Compatibility

To adjust binding capacity, simply replace the provided columns with the formats indicated below and follow their respective kit protocol.

Format	Zymo-Spin™ I & IC	Zymo-Spin™ II & IICR	Zymo-Spin™ V-E	Zymo-Spin™ I-96 Plate
Item Image				
Kit Name	RCC™-5	RCC™-25	RCC™-100	RCC™-96
Capacity	10 µg / prep.	50 µg / prep.	1 mg / prep.	10 µg / prep.
Elution Vol.	≥ 6 µl	≥ 25 µl	≥ 100 µl	≥ 10 µl
Column Cat. Nos.	C1003-50 , C1004-50	C1008-50 , C1078-50	C1024-25 , C1029-25	C2004



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Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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