ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

RNA Clean & Concentrator™-5

Catalog Nos. **R1013 & R1014** (supplied with DNase I)
R1015 & R1016

Highlights

- Quick, 5 minute recovery of ultra pure RNA (≥17 nt) from enzymatic reactions, aqueous phase (following Trizol® extraction) and other sources.
- High-quality RNA eluted in ≥6 µl is ready for reverse transcription, microarray, sequencing, etc.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

Notes:

[1] Before use, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1013, R1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1014, R1016).

[2] Prior to use, reconstitute DNase I as indicated on the vial prior to use. Store frozen aliquots.

[3] Compatible with: TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

Product Contents

RNA Clean & Concentrator™-5 (Kit Size)	**R1013** (50 Preps.)	**R1014** (200 Preps.)	**R1015** (50 Preps.)	**R1016** (200 Preps.)
RNA Binding Buffer	25 ml	100 ml	25 ml	100 ml
RNA Prep Buffer	25 ml	100 ml	25 ml	100 ml
RNA Wash Buffer[1] (concentrate)	12 ml	2x 24 ml	12 ml	2x 24 ml
DNase I[2] (lyophilized)	1	4	-	-
DNA Digestion Buffer	4 ml	16 ml	-	-
DNase/RNase-Free Water	4 ml	10 ml	4 ml	10 ml
Zymo-Spin™ IC Columns	50	200	50	200
Collection Tubes	50	200	50	200
Instruction Manual	1	1	1	1

Note - 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.

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

Specifications

- **Sample Sources** – DNase I treated RNA, *in vitro* transcription products, the aqueous phase following TRIzol®/chloroform or similar[3] extraction (page 4).
- **RNA Size Limits** – From 17 nt to ~23 kb.
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for reverse transcription, microarray, sequencing etc.
- **RNA Recovery** – Up to 10 µg RNA can be eluted into as little as ≥6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is optional but highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

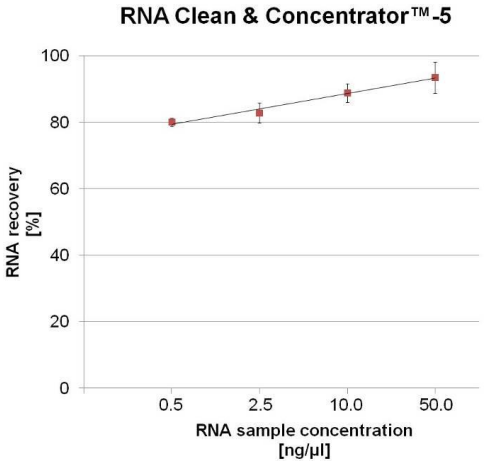
Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.
TRI Reagent®, TRIzol® and RNAzol® (Molecular Research Center, Inc.), QIAzol® (Qiagen GmbH), TriPure™ (Roche Diagnostics Operations, Inc.), TriSure™ (Bioline Ltd.), RNA*later*® (Ambion, Inc.).

Product Description

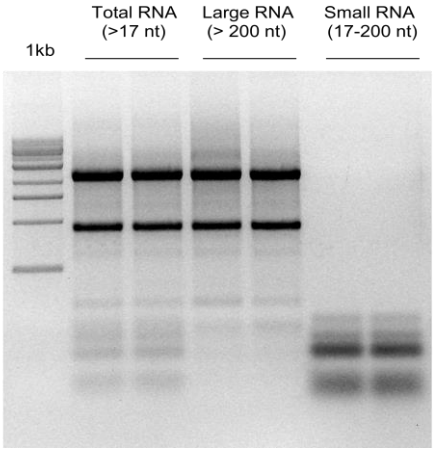
RNA Clean & Concentrator™-5 provides a simple and reliable method for the rapid preparation of up to 10 µg of high-quality RT-PCR-ready, DNA-free (R1013, R1014) RNA. This simple procedure is based on the use of a unique single-buffer system and Clean-Spin™ column technology that allows for selective recovery of total RNA (> 17 nt), 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™ IC Column** in as little as ≥6 µl of RNase-free water. The highly-concentrated, purified RNA is suitable for all subsequent analyses and molecular manipulations.

The entire procedure typically takes about 5 minutes.



Concentration of diluted RNA samples. RNA was eluted with 20 µl RNase-free water (n = 3, total input = 1 µg RNA).



Purification of small and large RNAs into separate fractions. RNA Clean & Concentrator™ allows for purification of total RNA (> 17 nt), large RNAs (> 200 nt), and/or small RNAs (17-200 nt).

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Note:

For purification of DNA see the **DNA Clean & Concentrator™-5** and **-25** (Catalog Nos. D4013, D4014, D4033, D4034).

Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Buffer Preparation

Before starting, add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1013, R1015) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1014, R1016).

Protocol

All centrifugation steps should be performed at 10,000 – 16,000 *x g.*
RNA species ≥17 nt will be recovered.
For DNA-free RNA, perform DNase I treatment prior or during the clean-up protocol (page 4).

1. Add 2 volumes **RNA Binding Buffer** to each sample[1] and mix.

   Example: Mix 100 µl buffer and 50 µl sample.

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

   Example: Add 150 µl ethanol.

3. Transfer the sample[2] to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.

4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.

5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.

6. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).

7. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Alternatively, for highly concentrated RNA use ≥6 µl elution.

   The eluted RNA can be used immediately or stored at -70°C.

Notes:

[1] Adjust the sample volume to 50 µl (minimum).

[2] To process samples >800 µl, **Zymo-Spin™** columns may be reloaded.

DNase I treatment

There are two methods of performing the DNase I digestion: (I) before the clean-up and (II) during the clean-up (in-column). Choose an appropriate method for your application below:

(I) Before clean-up

The DNase digestion procedure can be performed using the **DNase I Set** (E1010; provided with R1013, R1014)[1].

1. For each sample to be treated, prepare **DNase I reaction mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

RNA sample (≤10 µg)	
volume adjusted with water or TE buffer	40 µl
DNase I	5 µl
DNA Digestion Buffer	5 µl
	50 µl

2. Incubate at room temperature (20-30ºC) for 15 minutes. Then start with RNA purification (page 3, step 1).

(II) In-column

All centrifugation steps should be performed at 10,000 – 16,000 *x g.*

1. Following the RNA binding step (page 3, step 3), prewash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow through.
2. For each sample to be treated, prepare **DNase I reaction mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	35 µl

3. Add 40 µl reaction mix directly to the column matrix. Incubate at room temperature (20-30ºC) for 15 minutes. Then continue with RNA purification (page 3, step 4).

RNA purification from aqueous phase after TRIzol® extraction

1. Following Trizol®/chloroform or similar[2] extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided).
2. For each volume of the aqueous phase (as measured or estimated), add 1 volume ethanol (95-100%) and mix.
3. Then continue with purification (page 3, step 3).

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

[2] Compatible with: TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™ and other *acid-guanidinium-phenol* reagents.

Purification of small and large RNAs into separate fractions

All centrifugation steps should be performed at 10,000 – 16,000 *x g*.
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 sample and mix.

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

3. Transfer the mixture to the **Zymo-Spin™ Column** and centrifuge for 30 seconds.

Save the flow-through!

4. 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 for 30 seconds. Discard the flow-through.

   RNAs >200 nt
   are retained in the **column**.
   Continue with step 5.

5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
8. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Alternatively, for highly concentrated RNA use ≥6 µl elution.

   The eluted RNA can be used immediately or stored at -70°C.

Ordering Information

Product Description	Catalog No.	Kit Size
RNA Clean & Concentrator™-5	R1015 R1016	50 Preps. 200 Preps.
RNA Clean & Concentrator™-5 with DNase I Set	R1013 R1014	50 Preps. 200 Preps.
RNA Clean & Concentrator™-25	R1017 R1018	50 Preps. 100 Preps.
RNA Clean & Concentrator™-100	R1019	25 Preps.
ZR-96 RNA Clean & Concentrator™	R1080	2x 96 Preps.

For Individual Sale	Catalog No.	Amount
RNA Binding Buffer	R1013-2-25 R1013-2-50 R1013-2-100 R1013-2-1000	25 ml 50 ml 100 ml 1000 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
DNase I Set DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set

DNA PURIFICATION

What is Clean-Spin™ Technology?

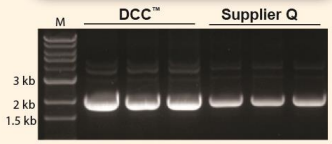
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

Product	Size (Cat. No.)
DNA Clean & Concentrator™-5	50 Preps. (D4013) 200 Preps. (D4014)
ZR-96 DNA Clean & Concentrator™-5	2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024)
Genomic DNA Clean & Concentrator™	25 Preps. (D4010) 100 Preps. (D4011)



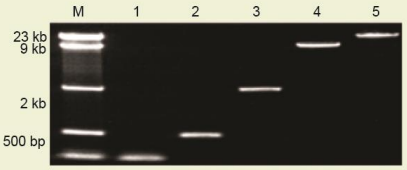
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

Product	Size (Cat. No.)
Zymoclean™ Gel DNA Recovery Kit	50 Preps. (D4001) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli.*
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

Easy, Pellet-free Procedure: Add Lysis Buffer ***Directly*** to Bacterial Culture

Product	Size (Cat. No.)
Zyppy™ Plasmid Miniprep Kit	50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

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RNA PURIFICATION

What is Clean-Spin™ Technology?

The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

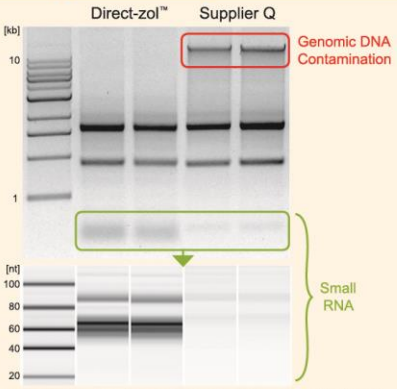
Get RNA *directly* from TRIzol® without phase separation

Direct-zol™ RNA

- ✓ For purification of high-quality small and large RNA *directly* from TRIzol®, TRI Reagent®, or similar.
- ✓ Bypasses phase separation and precipitation procedures allowing for unbiased recovery of miRNA

Product	Size (Cat. No.)
Direct-zol™ RNA MiniPrep	50 Preps. (R2050) 50 Preps. (R2051)* 200 Preps. (R2052) 200 Preps. (R2053)*
96-well and MagBead formats also available!	

DNase I included in all kits.
* Supplied with *TRI-Reagent®*



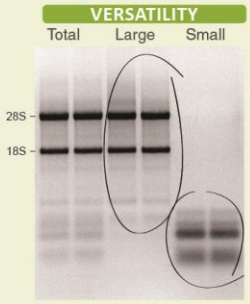
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

Isolate DNA-free RNA from 1 to 10^7 cells in minutes

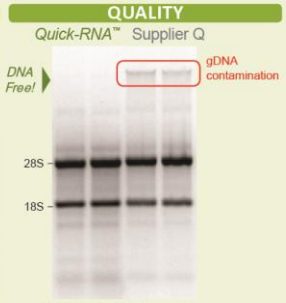
Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10^7 cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

Product	Size (Cat. No.)
Quick-RNA™ MicroPrep	50 Preps. (R1050) 200 Preps. (R1051)
Quick-RNA™ MiniPrep	50 Preps. (R1054) 200 Preps. (R1055)
ZR-96 *Quick-RNA*™	2 x 96 Preps. (R1052) 4 x 96 Preps. (R1053)



Isolate total, large, or small RNA with the *Quick-RNA*™ kit.



RNA is DNA-free using the *Quick-RNA*™ kit

Purify RNA from enzymatic and labeling reactions in 5 minutes

RNA Clean & Concentrator™

- ✓ Recover ultra-pure RNA in small (≥6 µl) elution volumes.
- ✓ Compatible with TRIzol®, phenol, choloform, and RNase inhibitors (RNAlater®).
- ✓ RNA is ideal for RT-PCR, q-PCR, hybridization, arrays, RNA interference, etc.

Product	Size (Cat. No.)
RNA Clean & Concentrator™-5	50 Preps. (R1015) 200 Preps. (R1016)
RNA Clean & Concentrator™-25	50 Preps. (R1017) 100 Preps. (R1018)
ZR-96 RNA Clean & Concentrator™	2x96 well plates (R1080)
DNA-Free RNA Kit™	50 Preps. (R1013) 200 Preps. (R1014)

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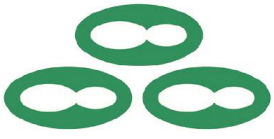

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