ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ MicroPrep

Catalog Nos. **R1050 & R1051**

Highlights

- High-quality total RNA from a wide range of samples – single to 10^6 cells.
- Isolate small and large RNAs into separate fractions (optional).
- *DNA-free* RNA for use in any downstream application. *DNase I included.*

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please contact us.

Product Contents

***Quick-RNA*™ MicroPrep** (Kit Size)	**R1050** (50 Preps.)	**R1051** (200 Preps.)
RNA Lysis Buffer	50 ml	2x 100 ml
RNA Prep Buffer	25 ml	100 ml
RNA Wash Buffer[1] (concentrate)	24 ml	2x 48 ml
DNase/RNase-Free Water	6 ml	2x 10 ml
DNase I Set[2] DNase I (250 U) & 10x DNase I Reaction Buffer (1 ml)	1 set	4 sets
Zymo-Spin™ IC Columns	50	200
Collection Tubes	50	200
Instruction Manual	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. Store reconstituted DNase I at -20 ºC.

[1] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate before use.

[2] Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** (E1009) at 1 U/µl. Mix by gentle inversion. Store aliquots at -20 ºC.

Specifications

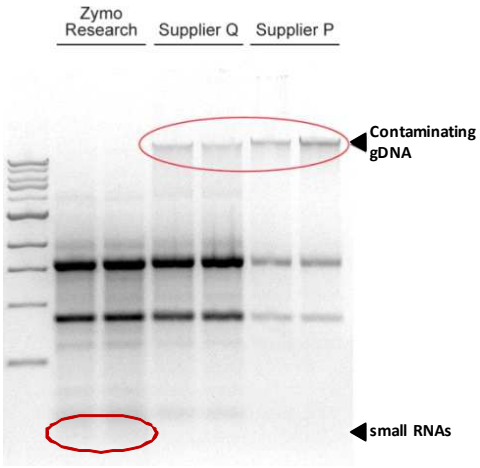
Some difficult-to-lyse samples may require mechanical or enzymatic homogenization. For assistance, contact us at tech@zymoresearch.com.

For 10^2 to 10^7 cells, use the ***Quick*-RNA™ MiniPrep** (Cat. Nos. R1054, R1055).

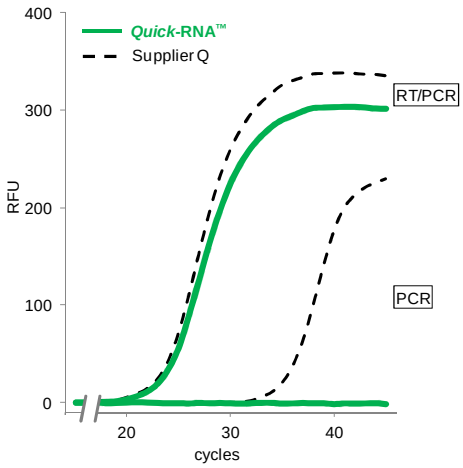
- **Sample Sources** – Cells or tissue samples, yeast, plant, bacteria, buccal cells, buffy coat, plasma, serum, and other biological liquids. *Compatible with RNA Shield™ (Cat. No. R1100) and RNAlater™.*
- **Sample Storage** – Samples homogenized in RNA Lysis Buffer are stable and can be stored frozen prior to purification.
- **Sample Size** – Up to 10^6 cells or 5 mg tissue.
- **RNA Purity** – High quality RNA (A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8) suitable for all downstream RNA-based manipulations.
- **RNA Recovery** – Up to 10 µg RNA can be eluted into ≥6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored frozen. RNase inhibitors can be included for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Product Description

The ***Quick-RNA***™ **MicroPrep** kit is an innovative product designed for the easy, reliable, and rapid isolation of DNA-free RNA from a wide range of cell *(up to 10^6)* and tissue samples *(up to 5 mg)*. The procedure combines a unique buffer system with *Fast-Spin* column technology to yield high quality total RNA *(including small RNAs 17-200 nt)* in about 10 minutes.
The procedure is simple: Add the provided **RNA Lysis Buffer** to a sample, then purify the RNA using the **Zymo-Spin**™ **Columns**. The result is highly-concentrated, *DNA-free* RNA that is suitable for subsequent RNA-based methods including RT-PCR, hybridization, sequencing *etc*. In addition, the kit can be used for enrichment of small and large RNAs in two separate fractions (see Appendix, page 5).



The ***Quick-RNA***™ **MicroPrep** yields high quality total RNA. High levels of genomic DNA contamination are present in the preps from Suppliers Q & P but not with the ***Quick-RNA***™ **MicroPrep**. Total RNA was isolated from human epithelial cells (sans DNase treatment).



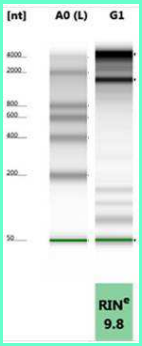
RNA isolated with the ***Quick-RNA***™ **MicroPrep** is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10^6 human epithelial cells (with in-column DNase treatments for both kits). Each amplification curve represents an average of three independent isolation experiments.

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

Notes:

Use the **Direct-zol**™ **RNA MiniPrep** (Cat. Nos. R2050, R2051, R2052, R2053) for isolation of RNA directly (without phase separation) from samples in Trizol®, *etc.*

Use the **RNA Shield**™ (Cat. Nos. R1100-50, R1100-250) for safe sample storage and transport at ambient temperatures.



The ***Quick-RNA***™ kits yield high quality RNA with high "RNA Integrity Numbers" (2200 TapeStation, Agilent).

Ensure the RNA isolation procedure is performed in an RNase-free environment.

Buffer Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1050) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1051).
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** (E1009) at 1 U/µl. Mix by gentle inversion. Store aliquots at -20 ºC.

Protocols

The RNA isolation consists of three steps: (I) Sample Lysis/Homogenization, (II) Sample Clearing and (III) RNA Purification.

All steps should be performed at room temperature (20-30 ºC).

I. Sample Lysis/Homogenization

Notes:

Samples homogenized in **RNA Lysis Buffer** can be stored frozen for processing at a later time.

Recommended **RNA Lysis Buffer** volumes

RNA Lysis Buffer	100 µl	300 µl
Cells	Up to 10^5	Up to 10^6
Tissue	-	Up to 5 mg

Adherent Cells

Lyse cells directly in the culture container by removing liquid medium and adding **RNA Lysis Buffer** directly to the monolayer. Remove cells from culture surface by pipetting, scraping, *etc.*

Cells in Suspension

Pellet cells *(≤500 x g)*, remove the supernatant completely then resuspend the cell pellet in **RNA Lysis Buffer**. Vortex briefly.

Tissue and Tough-to-Lyse Samples

Fresh or frozen tissue (animal, plant, insect, yeast or bacteria) can be mechanically homogenized (**ZR BashingBead™ Lysis Tubes**, Dounce or similar) directly in the **RNA Lysis Buffer**. Alternatively, tough-to-lyse samples can undergo enzymatic treatment (Proteinase K (Appendix C, page 5) – tissue, Zymolyase - yeast) prior to adding the **RNA Lysis Buffer**.

ZR Bashing Bead™ Lysis Tubes are available separately (Cat. Nos. S6002, S6003).

Processing plant tissue and other samples containing polyphenolics, humic acids, melanin, *etc.* may require use of the **OneStep™ PCR Inhibitor Removal Kit** (Cat. No. D6030).

For whole-blood samples, use the **ZR Whole-Blood RNA MiniPrep™** (Cat. Nos. R1020, R1021).

Liquids

DNase-treated RNA, labeling reactions, aqueous phase (following Trizol® extraction), and biological liquids can be processed directly by adding 4 volumes of **RNA Lysis Buffer** to each volume of sample (4:1) then mixing well. Plasma and serum samples require digestion with Proteinase K (Appendix C, page 5) before processing.

Samples in RNA Shield™

Bring samples homogenized and stored in **RNA Shield™** to room temperature (20-30 ºC) before proceeding with *Sample Clearing* step.

Samples in RNA*later*™

To process cells or liquids in RNA*later*™ (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **RNA Lysis Buffer** (4:1) and mix.

Note: Alternatively, remove the RNA*later*™ then proceed with the recommended *Sample Lysis/Homogenization* (above) according to the sample type.

II. Sample Clearing

The following is recommended for cells and tissue (animal/plant) but can be omitted for cell-free liquids and low input samples (≤10^5 cells).

For particulate removal, centrifuge lysates at ≥12,000 x *g* for 1 minute. Then transfer the supernatant into an RNase-free tube (*not provided*).

III. RNA Purification

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

1. Add 1 volume ethanol (95-100%) to the sample in **RNA Lysis Buffer** (1:1). Mix well.
2. Transfer the mixture to a **Zymo-Spin™ IC Column**[1] in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.
3. **In-column DNase I Treatment** (optional)

   This step can be used for trace DNA removal.

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

   b. Add 50 µl of the **DNase I reaction mix** *(see below)* directly to the column matrix. Incubate the column at room temperature (20-30 ºC) for 15 minutes, then centrifuge for 30 seconds.

   For each sample to be treated, prepare **DNase I reaction mix**[2] in an RNase-free tube (not provided). Mix gently.

DNase I	5 µl (1 U/µl)
10X DNase I Reaction Buffer	5 µl
RNA Wash Buffer (with ethanol added)	40 µl

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** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer.
7. Place the column into an RNase-free tube *(not provided)*. Add ≥6 µl **DNase/RNase-Free Water**[3] directly to the column matrix, then centrifuge at top speed for 30 seconds.

   Eluted RNA can be used immediately or stored frozen.

Notes:

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

[2] When adjusting the volume and composition, make sure the **RNA Wash Buffer** in the **DNase I reaction mix** remains at 80% (v/v).

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.

[3] To maximize RNA yield, preheat the DNase/RNase-Free Water to 95º C, increase the elution volume and/or repeat the elution.

Appendices

A. Purification of Small and Large RNAs into Separate Fractions

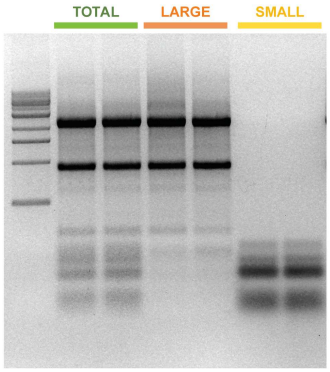
The ***Quick-RNA***™ **MicroPrep** also allows for purification of small (17-200 nt) and large RNAs (>200 nt) into separate fractions. This procedure is compatible only with cell inputs (up to 10^6) or previously isolated RNA. Processing requires two Zymo-Spin™ IC columns per prep.

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

1. Prepare a modified **RNA Lysis Buffer** with the addition of 1 volume ethanol (95-100%) (1:1). Mix. Add 2 volumes of this modified buffer to each volume of RNA sample[1] (2:1) or 300 µl buffer to pelleted animal cells. Mix well. Do not centrifuge the sample.
2. Transfer the mixture to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge for 30 seconds. *Important: Save the flow-through!*

   To purify large RNAs (>200 nt) from this column, continue to *RNA Purification* (page 4, step 3).
3. To the **flow-through**, add 1 volume ethanol (95-100%) (1:1) and mix well.
4. Transfer the mixture to a **new Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through.

   To purify small RNAs (17-200 nt) from this column, continue to *RNA Purification* (page 4, step 3)[2].



Total RNA (>17 nt), large (>200 nt) or small RNAs (17-200 nt) are effectively partitioned and purified with the ***Quick-RNA***™ kit.

B. RNA Clean-Up

The ***Quick-RNA***™ **MicroPrep** can also be used for RNA clean-up. To clean RNA following DNase treatment, labeling reactions or from an aqueous phase (following acid-guanidinium-phenol extractions); simply add 4 volumes **RNA Lysis Buffer** and proceed with the *RNA Purification (page 4)*.

C. Proteinase K Digestion

Tissue, Plasma, Serum *(RNase-free water for solid tissue up to 5 mg)*	95 µl
2X Digestion Buffer	95 µl
Proteinase K	≥6 U[3]

Prepare a Proteinase K reaction mix (see example above, scale-up as necessary). Incubate at 55°C for 30 minutes (plasma, serum) or 1-3 hours (tissue). Then add 4 volumes **RNA Lysis Buffer** and proceed to *Sample Clearing* (page 4).

Notes:

[1] Minimal recommended sample volume is 50 µl. Adjust samples <50 µl with RNase-free water.

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

[3] One unit (1 U) of enzyme will hydrolyze urea-denatured hemoglobin to produce 1.0 µmole of tyrosine per minute at pH 7.5 at 37°C.

Ordering Information

Product Description	Input	Binding	Catalog No.	Kit Size
***Quick-RNA*™ MicroPrep**	~1-10^6 cells	~10 µg	R1050 R1051	50 Preps. 200 Preps.
***Quick-RNA*™ MiniPrep**	~10^2-10^7 cells	~100 µg	R1054 R1055	50 Preps. 200 Preps.
***Quick-RNA*™ MidiPrep**	~10^6-10^8 cells	~1 mg	R1056	25 Preps.
ZR-96 *Quick-RNA*™	~1-10^6 cells	~10 µg/well	R1052 R1053	2x 96 Preps. 4x 96 Preps.

For Individual Sale	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 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
DNase I (lyophilized) (250 U supplied with 10x DNase I Reaction Buffer)	E1009	1 set
Zymo-Spin™ IC Column	C1004-50-G C1004-250-G	50 250
Collection Tube	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-6 W1001-10	1 ml 6 ml 10 ml

Related Products

Product	Description	Prep/Format	Catalog
	RNA Clean-Up		
RNA Clean & Concentrator™-5	Cleanup and concentration of modified, labeled, impure, diluted, DNase treated RNA (≥ 17nt) and purification of RNA from aqueous phase of organic extracts. *Note: DNA-free RNA Kit™ includes DNase I*	50/column 200/column	R1015 R1016
RNA Clean & Concentrator™-25		50/column 100/column	R1017 R1018
RNA Clean & Concentrator™-100		25/column	R1019
ZR-96 RNA Clean & Concentrator™		2x 96/plate	R1080
DNA-Free RNA Kit™		50/column 200/column	R1013 R1014
Oligo Clean & Concentrator™	Cleanup and concentration of RNA and/or DNA oligos. Good for clean-up of miRNAs and siRNAs.	50/column 200/column	D4060 D4061
ZR-96 Oligo Clean & Concentrator™		2x 96/plate 4x 96/plate	D4062 D4063
ssDNA/RNA Clean & Concentrator™	Separation of short ssRNA and ssDNA (up to 200 nt) from double stranded species.	20/column 50/column	D7010 D7011
Zymoclean™ Gel RNA Recovery Kit	Recovery of RNA from agarose gels.	50/column	R1011
ZR small-RNA™ PAGE Recovery Kit	Small RNA (> 17nt) from polyacrylamide gels.	20/column	R1070
***OneStep*™ PCR Inhibitor Removal Kit**	Removal of polyphenolics, humic/fulvic acids, tannins, melanin etc. from RNA.	50/column	D6030
***OneStep*™-96 PCR Inhibitor Removal Kit**		2x 96/plate	D6035
	RNA from Samples in TRI Reagent (Small RNA Recovery)		
Direct-zol™ RNA MiniPrep	RNA (>17 nt) from TRI Reagent®, TRIzol®, and all other acid-guanidinium-phenol based reagents without phase separation. DNase I included.	50/column 200/column	R2050 R2052
Direct-zol™ RNA MiniPrep w/ TRI Reagent		50/column 200/column	R2051 R2053
Direct-zol™-96 RNA		2x 96/plate 4x 96/plate	R2054 R2056
Direct-zol™-96 RNA w/ TRI Reagent		2x 96/plate 4x 96/plate	R2055 R2057
Direct-zol™-96 MagBead RNA	RNA (>17 nt) from TRI Reagent®, TRIzol®, and all other acid-guanidinium-phenol based reagents without phase separation. These kits are in a magnetic bead format that is adaptable for high-throughput and automated protocols. DNase I included.	2x 96/plate 4x 96/plate 8x 96/plate	R2100 R2102 R2104
Direct-zol™-96 MagBead RNA w/ TRI Reagent		2x 96/plate 4x 96/plate 8x 96/plate	R2101 R2103 R2105
	RNA from Cells & Tissue		
***Quick-RNA*™ MicroPrep**	Total RNA (>17 nt) from cultured cells, tissue samples, buccal cells, buffy coat, plasma, serum, and other biological liquids. DNase I included.	50/column 200/column	R1050 R1051
***Quick-RNA*™ MiniPrep**		50/column 200/column	R1054 R1055
***Quick-RNA*™ MidiPrep**		25/column	R1056
ZR-96 *Quick-RNA*™		2x 96/plate 4x 96/plate	R1052 R1053
ZR-*Duet*™ DNA/RNA MiniPrep	Parallel purification of DNA/RNA from cells.	50/column	D7001
Pinpoint™ Slide RNA Isolation System Kit I	RNA from fresh/frozen tissue sections.	50/column	R1003
Pinpoint™ Slide RNA Isolation System Kit II	RNA from paraffin-embedded (FFPE) tissue.	50/column	R1007
	RNA from Biological Liquids		
ZR Viral RNA Kit™	RNA (DNA) from body fluids (plasma, serum, CSF, urine).	50/column 200/column	R1034 R1035
ZR-96 Viral RNA Kit™		2x 96/plate 4x 96/plate	R1040 R1041
ZR Viral DNA/RNA Kit™		25/column 100/column	D7020 D7021
ZR Whole-Blood RNA MiniPrep™	RNA from whole blood or partitioned blood.	50/column 100/column	R1020 R1021
ZR Urine RNA Isolation Kit™	Cellular and endosomal RNA from urine.	20/column 50/column	R1038 R1039
	RNA from Tough-to-Lyse Samples		
ZR Fungal/Bacterial RNA MicroPrep™	RNA from bacteria, yeast, fungi; BashingBead™ lysis.	50/column	R2010
ZR Fungal/Bacterial RNA MiniPrep™		50/column	R2014
ZR Plant RNA MiniPrep™	RNA from leaves, stems, buds, flowers, fruits, seeds, etc; BashingBead™ lysis, RT/PCR inhibitor removal.	50/column	R2024
ZR Tissue & Insect RNA MicroPrep™	RNA from insect, arthropod specimen and small tissue samples; BashingBead™ lysis.	50/column	R2030
ZR Soil/Fecal RNA MicroPrep™	RNA from soil, sludge, sediment, feces.	50/column	R2040
YeaStar RNA Kit™	RNA from yeast strains susceptible to Zymolyase.	50/column	R1002
	RNA Sample Preservation and Storage		
RNA Shield™	Cells, biological liquid, tissue storage and RNA purification.	50 ml 250 ml	R1100-50 R1100-250
RNA Shield™ Purification Kit (RNA Shield™ reagent included)		50/column	R1100
RNA Shield™ Purification Kit (RNA Shield™ reagent is <u>not</u> included)		50/column	R1101
	Enzymes and Markers		
DNase I w/ 10X Reaction Buffer	Lyophilized	250 U	E1009
ZR small-RNA™ Ladder	ssRNA (17, 21, 25, 29 nt)	10 µg	R1090