ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ Miniprep Kit

DNA & RNA from any sample

Highlights

- Spin-column purification of DNA and total RNA (including small/microRNAs) from cells and tissue.
- High-quality DNA & RNA are eluted in two separate fractions and are ready for any downstream application.

Catalog Numbers:
D7001

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

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Table of Contents

Product Contents

Quick-DNA/RNA™ Miniprep Kit	D7001 (50 prep)
DNA/RNA Lysis Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[1]	24 ml (x2) (concentrate)
DNase/RNase-Free Water	10 ml
Spin-Away™ Filters	50
Zymo-Spin™ IICR Columns	50
Collection Tubes	150
Instruction Manual	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
1 Before use, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Cells (animal, gram(-) bacteria), soft and easy-to-lyse tissue, samples in **DNA/RNA Shield™** or other preservation reagents, and enzymatic reactions (e.g., DNase I treated, Proteinase K treated). Not compatible with whole blood[1] and urine[1] samples.

- **Size –** Genomic DNA (≥ 40 kb), mitochondrial and viral DNA (if present) and total RNA including small/microRNAs (≥ 17 nt).

- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. DNA & RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity – Spin-Away™ Filter** (yellow) **and Zymo-Spin™ IICR Column** yield up to 100 µg DNA and 50 µg RNA, respectively.

- **Compatibility –** For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®) and RNAlater™.

- **Elution Volume –** ≥ 25 µl **DNase/RNase-Free Water.**

- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex.

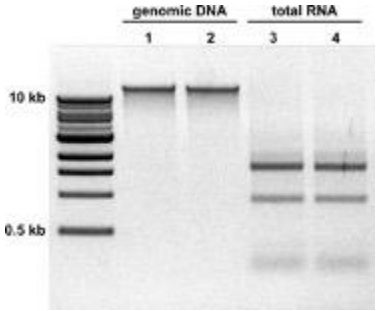
1 For DNA/RNA purification from whole-blood and urine, see the Quick-DNA/RNA Plus Kit (D7003, D7005) or the Quick-DNA/RNA MagBead Kit (R2130, R2131).

Product Description

The **Quick-DNA/RNA™ Miniprep Kit** provides a quick method for the isolation of high-quality genomic DNA and total RNA from cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue. Enrichment of small RNAs (e.g., tRNAs, microRNAs) can be recovered following a simple adjustment within the RNA isolation protocol – no extra steps required!

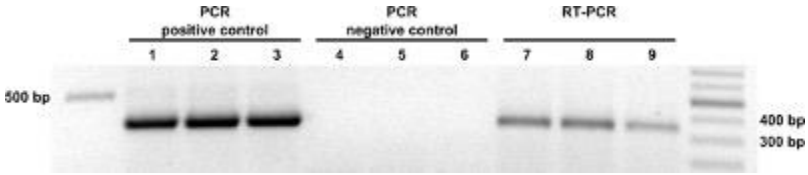
The procedure uses unique spin-column technology that results in high-quality DNA and total RNA (including small RNAs 17-200 nt) that are ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-quality DNA & RNA from cells



Genomic DNA (lane 1, 2) and total RNA (lane 3, 4) isolated from human epithelial cells (HCT116) with the **Quick-DNA/RNA™ Miniprep Kit**.

DNA & RNA is ready for any downstream application



PCR amplification of β-actin transcript (353 bp fragment shown) following DNA and RNA isolation from human epithelial cells (HCT 116) with the **Quick-DNA/RNA™ Miniprep Kit**: PCR positive control (DNA template; lane 1, 2, 3), PCR negative control (RNA template; lane 4, 5, 6), RT-PCR (lane 7, 8, 9).

Input Capacity and gDNA/Total RNA Yield

Input	Average gDNA Yield	Average RNA Yield	Kit Capacity
Cells	4 µg (per 10^6 cells)	10 µg (per 10^6 cells)	Up to 10^7
HeLa	6 µg	15 µg	
High Yield Tissue[1,2 (mouse)]	≥ 30 µg (per 10 mg)	≥ 30 µg (per 10 mg)	Up to 20 mg
Spleen	50-70 µg	30-50 µg	
Liver	15-30 µg	40-60 µg	
Low Yield Tissue[1,2 (mouse)]	≤ 30 µg (per 10 mg)	≤ 30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	5-15 µg	
Muscle	5-15 µg	5-20 µg	
Lung	15-30 µg	10-20 µg	
Intestine	15-30 µg	10-30 µg	
Kidney	15-30 µg	20-30 µg	
Whole Blood[1,3]	(per 1 ml)	(per 1 ml)	Up to 3 ml
Porcine	5-10 µg	10-20 µg	
Human	2-5 µg	2-10 µg	

1 Recommended: For protein-rich samples (e.g., tissue, blood cells, etc.) stored in DNA/RNA Shield™ (#R1100, sold separately), Proteinase K treatment can be performed using Proteinase K Set (#D3001-2-5, #D2001-2-20; sold separately) and PK Digestion Buffer (#R1200-1-5, #R1200-1-20; sold separately), see page 9.
2 Yield from tissue can vary due to other factors (i.e., organism type, physiological state, and growth conditions.
3 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) DNA & RNA Purification.

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.

(II) Sample Preparation

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

Samples stabilized and stored in DNA/RNA Shield™ (cells, tissue, etc.)

1. If frozen, thaw homogenized sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex.
2. Add an equal volume of **DNA/RNA Lysis Buffer** (1:1), mix well and proceed to purification, page 7.

Cells & Tissue (mammalian)

1. **Cells**:
   a. If in suspension[1], pellet by centrifugation (≤ 500 x g for 1 minute), remove supernatant and resuspend cell pellet in **DNA/RNA Lysis Buffer** (see table below). Proceed to purification, page 7.

   b. If adherent, remove liquid media from the culture container. Then add **DNA/RNA Lysis Buffer** directly to the monolayer (see table below). Remove cells from the culture surface by scraping, pipetting, etc. Proceed to purification, page 7.

Cells	Gram(-) Bacteria	Add DNA/RNA Lysis Buffer
≤ $5x10^6$	≤ 10^8	≥ 300 µl
$5x10^6$ - 10^7	≤ $5x10^8$	≥ 600 µl

2. **Tissue**[2]:
   a. Submerge an appropriate amount of fresh or frozen sample (see table below) into **DNA/RNA Lysis Buffer** and homogenize[3,4].

Tissue	Add DNA/RNA Lysis Buffer
High-yield (≤ 25 mg) Low-yield (≤ 50 mg)	≤ 600 µl

   b. To remove particulate debris, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided). Proceed to purification, page 7.

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1 If liquid/media cannot be removed, add ≥ 3 volumes **DNA/RNA Lysis Buffer** to 1 volume liquid sample (3:1) and mix well. Proceed to purification, page 7.
2 For examples of sample type input and average yield, see chart on page 4.
3 For efficient homogenization, bead beat samples with ZR BashingBead Lysis Tubes (S6012, S6003), sold separately. See Appendices (page 10) for bead beating parameters. Other types of homogenization can include mortar/pestle, dounce, syringe or tissue grinder, etc.
4 If no method of homogenization, alternatively tissue samples collected and stored in DNA/RNA Shield™ can be Proteinase K treated. See Appendices (page 9).

(III) DNA and RNA Purification (in two separate fractions)

✓ Perform all steps at room temperature and centrifuge at 10,000-16,000 x g for 30 seconds, unless specified.

1. Transfer the sample into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge. Save the flow-through for RNA purification and the filter for DNA purification!

DNA Purification
(DNA is in the filter.)

2a. Transfer the **Spin-Away Filter**[1] (**yellow**) into a new **Collection Tube**.

RNA Purification
(RNA is in the flow-through.)

2b. Add 1 volume ethanol (95-100%) to the flow-through and mix well.

Example: Add 300 µl ethanol to 300 µl flow-through.

Then transfer the mixture into a **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

At this point, **DNase I** treatment (in-column) can be performed (see page 9).

3. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

4. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 400 µl **DNA/RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

6a. To elute DNA, add 100 µl **DNase/RNase-Free Water** directly to the column matrix, let it stand for 2-5 minutes and centrifuge.

Alternatively, for highly concentrated DNA use ≥ 50 µl elution.

6b. To elute RNA, add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥ 25 µl elution.

The eluted DNA/RNA can be used immediately or stored frozen.

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1 To process samples > 700 µl, columns may be reloaded.
2 Optional: At this point, proteins can be purified from the flow-through (page 10).

Appendices

Sample stabilization and storage in DNA/RNA Shield™

Liquid samples (e.g., whole-blood): Add 3 volumes **DNA/RNA Shield™** (1X) to 1 volume sample (3:1). Mix well.

Solid samples (e.g., pelleted cells, tissue): Submerge sample (not to exceed 10% (v/v or w/v)) in **DNA/RNA Shield™** (1X) and homogenize (see Appendices, page 10).

Store samples in **DNA/RNA Shield™** at ambient temperature for ≥ 1 month or long term at frozen temperature. DNA/RNA Shield™ is directly compatible with most guanidinium-based extraction methods (e.g., no need to remove reagent from the lysed/homogenized sample).

Samples in RNAprotect, Allprotect, RNAlater, UTM/VTM, saline or PBS

- ✓ RNAProtect®, Allprotect®: Add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume of liquid sample (3:1). Mix well and/or homogenize base on sample type (see Sample Preparation, page 6). Then, proceed to purification, page 7.
- ✓ RNAlater™:
  a. Cells - Pellet[1] by centrifugation at up to 5,000 x g and remove RNAlater (supernatant). Proceed to Sample Preparation, page 6.
  b. Tissue - Transfer into a new tube with forceps and remove any excess RNAlater™. Proceed to Sample Preparation, page 6.

  Alternatively, for liquid samples from which RNAlater cannot be removed, add 1 volume of nuclease-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **DNA/RNA Lysis Buffer** to 1 volume sample/water (or PBS) mixture (4:1). Mix again and proceed to Total RNA Purification, page 9.
- ✓ Swab samples in UTM®/VTM®, saline or PBS: Remove swab and add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume sample (3:1). Mix well and proceed to purification, page 7.

  Optional: To inactivate pathogens, store at room temperature prior to purification, add 1 volume **DNA/RNA Shield™** (2X concentrate) to 1 volume liquid sample (1:1) and mix well. Then proceed to Sample Preparation, Samples in **DNA/RNA Shield™**, page 6.

Liquids/Reaction Clean-up (DNase I treated RNA, in vitro transcriptions, etc.)

Add 150 µl **DNA/RNA Lysis Buffer** to a ≥ 50 µl liquid sample (3:1) and mix well. Proceed to purification, page 7, step 2b.

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1 Different cells may react differently to centrifugation forces, and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNAlater™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting (e.g., 500 x g).

(Appendices continued)

DNase I Treatment (in-column)

✓ For DNA-free RNA, **DNase I** treatment can be performed using **DNase I Set** (E1010; 50 reactions) and DNA/RNA Wash Buffer (concentrate) (D7010-3-6), sold separately.

1. Following RNA binding step (page 7, step 2b), add 400 µl **DNA/RNA Wash Buffer** to the column, centrifuge and discard the flow-through.
2. To treat each sample, prepare **DNase I Reaction Mix** (see table below) in a nuclease-free tube (not provided) and invert gently to mix.
3. Add 80 µl directly into the column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification protocol (page 7, step 3).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/µl)[1]	5 µl
DNA Digestion Buffer	75 µl

Proteinase K Treatment

✓ Proteinase K treatment (optional) can be performed on protein-rich samples stored in **DNA/RNA Shield™** (#R1100, sold separately) (e.g., tissue, blood cells, etc.), using **Proteinase K Set** (#D3001-2-5, #D2001-2-20; sold separately) and **PK Digestion Buffer** (#R1200-1-5, #R1200-1-20; sold separately).

1. For every 300 µl of **DNA/RNA Shield™** sample, add 15 µl **Proteinase K** and 30 µl **PK Digestion Buffer**. Mix and incubate at room temperature (20-30°C) for ≥30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
2. To remove particulate debris from homogenized tissue, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided).
3. Add **DNA/RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 7.

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

(Appendices continued)

Protein Purification: Acetone Precipitation of Proteins

✓ After the RNA binding to the column (page 7, step 2b), the protein content in the flow-through can be purified:

1. Add 4 volumes of cold acetone (-20°C) to flow-through (4:1) and mix.
2. Incubate the samples for 30 minutes on ice.
3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
5. Air-dry the protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

Homogenization with ZR BashingBead Lysis Tubes

✓ Recommended for complete and efficient homogenization of tough-to-lyse samples (e.g., tissue, plant, seed, microbes, etc.). Lysis tubes sold separately.

✓ For high-speed (e.g., MP Bio FastPrep-24, Bertin Precellys) and low-speed (e.g., Vortex Genie) homogenizers, bead-beating time optimization may be required.

Input	Tissue		Microbes
	Mammalian	Plant/Seed or Insect	Bacteria, Swab, Yeast, Stool/Soil
Cat. no. (lysis bead size)	S6003 (2.0 mm)	S6003 (2.0 mm)	S6012 (0.5 mm and 0.1 mm)
High-speed	30-60 sec	3-5 min	30-60 sec
Low-speed	3-5 min	15-20 min	5-10 min

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ Miniprep Kit	D7001	50 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Lysis Buffer	D7001-1-50 D7001-1-200	50 ml 200 ml
DNA/RNA Prep Buffer	D7010-2-25 D7010-2-50	25 ml 50 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-12 D7010-3-24	12 ml 24 ml
DNase/RNase-Free Water	W1001-6 W1001-30	6 ml 30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IICR Columns	C1078-50	50
Collection Tubes	C1001-50	50

Complete Your Workflow

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

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

✓ For isolation of DNA/RNA from any sample:

Quick-DNA/RNA Plus kits	
Microprep Plus #D7005	From 1 cell and up
MagBeads #R2130	Automatable (Tecan, Hamilton, Kingfisher, etc.)

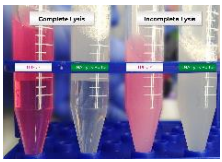
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

✓ For NGS:

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

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
Precipitation, viscous lysate	Incomplete lysis and/or high-mass input: - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)	Sample handling: - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. Incomplete lysis and/or cellular debris: - Increase the volume DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge and pellet any cellular debris then process the cleared lysate.
Low yield	Sample input: - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer. High-protein content (blood, plasma/serum, etc.) - Perform Proteinase K treatment to the sample prior to purification. See Appendices.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 9) or perform DNase I treatment post-purification, then re-purify the treated sample. - For future preps, increase the volume of DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
RNA degradation	To prevent RNA degradation: - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or DNA/RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

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