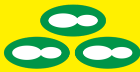

ZYMO RESEARCH

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 is eluted in two separate fractions and is ready for any downstream application.

Catalog Numbers:
D7001

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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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.
Before use:
1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Specifications

- **Sample Sources –** Cells (animal, buccal, buffy coat, gram(-) bacteria) and soft, easy-to-lyse tissue, plasma, serum, etc. Not compatible with whole-blood[1] and urine[2] 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. Trace DNA can be removed by DNase I digestion (page 8).

- **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.

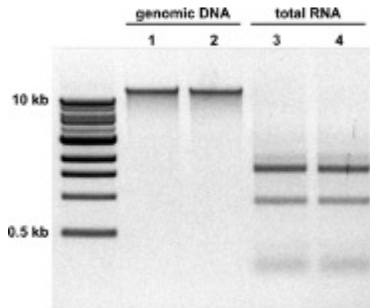
1 For DNA/RNA purification from whole-blood, see the Quick-DNA/RNA Plus Kit (D7003, D7005).
2 For urine, DNA/RNA can be isolated with the Quick-DNA/RNA MagBead Kit (R2130, R2131).

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!

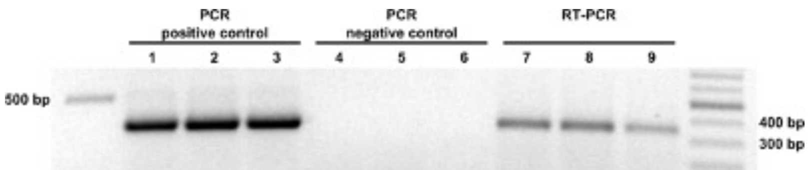
The procedure uses unique spin-column technology that results in high-quality DNA and total RNA (including small RNAs 17-200 nt) and is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

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).

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™

If frozen, thaw homogenized sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex. Proceed to the appropriate procedure below based on sample type (omit the step involving the addition of DNA/RNA Shield™).

Cells

a. **Samples in DNA/RNA Shield™**: Add an equal volume of **DNA/RNA Lysis Buffer** (1:1) and mix well.

b. **To pellet cells**: Centrifuge liquid sample at ≤ 500 x g for 1 minute and remove the supernatant. Then resuspend the cell pellet in **DNA/RNA Lysis Buffer** (see table below).

c. **Adherant cells**: 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, scraping, etc.

d. **Cells in suspension**: Add ≥ 3 volumes **DNA/RNA Lysis Buffer** to 1 volume of liquid sample and mix well.

Mammalian	Gram(-) bacteria	Add DNA/RNA Lysis Buffer
$\leq 5\times10^{6}$	$\leq 10^{8}$	≥ 300 µl
5×10^{6} - 10^{7}	$\leq 5\times10^{8}$	≥ 600 µl

Proceed to purification, page 6.

Tissue[1]

≤ 50 mg low yield tissue (or ≤ 25 mg high yield tissue) can be mechanically homogenized in ≥ 600 µl **DNA/RNA Lysis Buffer** with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating (recommended). To remove particulate debris from homogenate, centrifuge and transfer the supernatant into a new nuclease-free tube (not provided). Proceed to purification, page 6.

Recommended: Use ZR BashingBead Lysis Tubes (#S6003; sold separately) and a high-speed homogenizer (e.g., MP Bio FastPrep-24, Bertin Precellys) for 30-60 seconds.

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1 Tissue can be Proteinase K treated prior to adding DNA/RNA Lysis Buffer (page 9).

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

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

1. Transfer the sample lysed in **DNA/RNA Lysis Buffer** 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 (1:1) and mix well. Then transfer the sample into a **Zymo-Spin™ IICR Column**[1] in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

Optional: At this point, **DNase I** treatment (in-column) can be performed (page 8).

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 8).

Appendices

Samples stabilized and stored in DNA/RNA Shield™

Recommended: **DNA/RNA Shield™** effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume **DNA/RNA Shield™** (2X concentrate) and sample (1:1).
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in **DNA/RNA Shield™** (1X).

Mix well/homogenize sample prior to storage. Samples in **DNA/RNA Shield™** can be stored at ambient temperature ≥ month or long term at frozen temperature.

Samples in RNAProtect, All Protect, RNAlater, UTM/VTM, saline or PBS

✓ RNAProtect®, All Protect®: Add 3 volumes of **DNA/RNA Lysis Buffer** to 1 volume of liquid sample (3:1) and mix well and/or homogenize (e.g., see Tissue, page 5). Proceed to purification, page 6, step 2b.

✓ RNAlater™: Add 1 volume of RNase-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **DNA/RNA Lysis Buffer** (4:1) to 1 volume sample/water (or PBS) mixture. Mix again and proceed to purification, page 6, step 2b.

Alternatively, remove the RNAlater™, then proceed with Sample Preparation according to the sample type.

✓ 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 6, step 2b.

Optional: To inactivate, store and preserve at room temperature prior to purification, add 1 volume of 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 5.

(Appendices continued)

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

Add 150 µl **DNA/RNA Lysis Buffer** to ≥ 50 µl liquid sample (3:1) and mix well. Proceed to purification, page 6, step 2b.

In-Column DNase I Treatment

✓ 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); materials sold separately.

1. Following RNA binding step (page 6, step 2b), add 400 µl **DNA/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 a nuclease-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 purification protocol (page 6, step 3).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/ul)[1,2]	5 µl
DNA Digestion Buffer	75 µl

Protein Purification: Acetone Precipitation

✓ After the RNA binding to the column (page 6, step 2b), the protein content in the flow-through can be purified.

1. Add 4 volumes of cold acetone (-20°C) to the 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).

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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.

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

(Appendices continued)

Input Capacity and gDNA and 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] (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] (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[2]	(per 1 ml)	(per 1 ml)	Up to 3 ml
Porcine	5-10 µg	10-20 µg	
Human	2-5 µg	2-10 µg	

Proteinase K Treatment

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

1. For each sample to be treated, prepare **Proteinase K Reaction Mix** in a nuclease-free tube (not included) and mix by vortexing. Scale proportionally, if needed.

Proteinase K Reaction Mix	
Up to 5 mg animal tissue or 10^6 cells in **DNA/RNA Shield**™	300 µl
PK Digestion Buffer	30 µl
Proteinase K (reconstituted)[3]	15 µl

2. Incubate at room temperature (20-30°C) for 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.

3. Add 1 volume **DNA/RNA Lysis Buffer** to the treated sample (1:1) and mix. To remove particulate debris, centrifuge and transfer the supernatant into a new nuclease-free tube (not provided). Proceed to purification, page 6.

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1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state and growth conditions.
2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.
3 Reconstitute lyophilized **Proteinase K** (#D3001-2-20; 20 mg) with 1,040 µl **Proteinase K Storage Buffer** and mix by vortexing. Store frozen aliquots.

Ordering Information

Product Description	Catalog No.	Size
Quick-DNA/RNA™ Miniprep Plus Kit	D7003T D7003	10 preps. 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-G	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.)

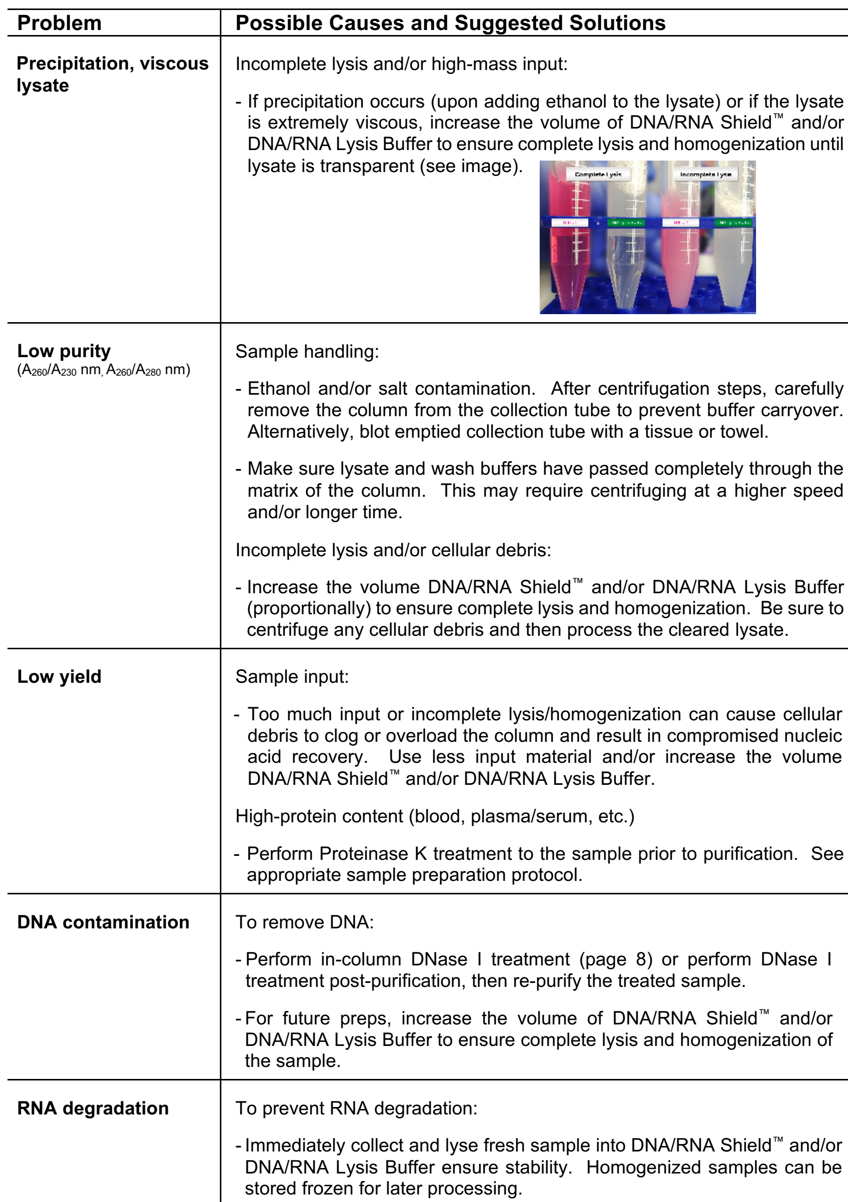
✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol® extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

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 any cellular debris and 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 appropriate sample preparation protocol.
DNA contamination	To remove DNA: - Perform in-column DNase I treatment (page 8) 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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