ZYMO RESEARCH

RNA Purification Made Simple™

ZR Urine RNA Isolation Kit™

RNA from urine

Highlights

- Quick, spin-column purification of total RNA from cells, biological sediment in urine, large volume liquid samples and suitable for isolation of RNA from microvesicles.
- Zymo-Spin column technology allow RNA to be eluted in ≥ 6 µl.
- RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

Catalog Numbers:
R1038, R1039

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

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Table of Contents

Product Contents

ZR Urine RNA Isolation Kit™	R1038 (20 prep)	R1039 (50 prep)
Urine RNA Buffer	20 ml	50 ml
RNA Prep Buffer	10 ml	25 ml
RNA Wash Buffer[1] (concentrate)	12 ml	24 ml
DNase/RNase-Free Water	1 ml	1 ml
ZRC GF™ Filter	20	50
Zymo-Spin™ IC Columns	20	50
Collection Tubes	20	50
Instruction Manual	1	1

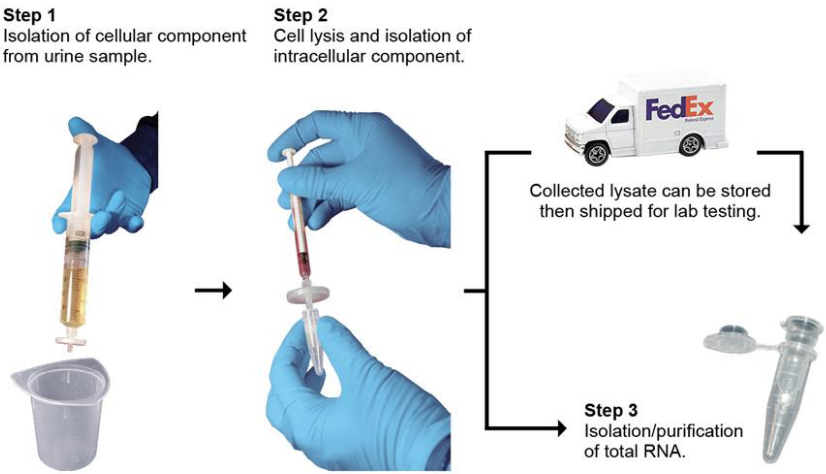
Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1038) or 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1039).

Specifications

- **Sample Sources –** Up to 30 ml urine (standard reaction) and other aqueous samples containing cells, biological sediment, microvesicle-associated RNA, etc.
- **Recovery –** Typically, 0.2 to 3.0 µg RNA per 30 ml urine sample.
- **Purity –** A_{260}/A_{280} & A_{260}/A_{230} > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc. Trace DNA can be removed by DNase I digestion (page 7).
- **Binding Capacity – Zymo-Spin™ IC Column** yield up to 10 µg RNA.
- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water.**
- **Equipment Needed (user provided) –** Microcentrifuge, vortex, syringes (i.e., 30 ml, 1 ml).

Product Description

The **ZR Urine RNA Isolation Kit™** is designed for the rapid isolation of total RNA from cells in urine samples. Cells from urine are separated using a syringe (not provided) and a uniquely-designed syringe filter. Cells are then lysed and total RNA is stabilized using a specially formulated **Urine RNA Buffer**. The collected lysate can then be used immediately or at a later time following transportation and/or storage. Also, this kit is ideal for direct isolation of RNA from microvesicles that may be recovered from urine filtrates. One-step RNA isolation occurs via matrix adsorption using **Zymo-Spin™ IC Columns**, then washed and eluted. High-quality, total RNA from urine samples is suitable for subsequent analyses of gene expression that include NGS, RT/qPCR, etc.



Protocol

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

(I) Buffer Preparation

- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate (R1038) or 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1039).

(II) Sample Preparation

- ✓ Perform all steps at room temperature, unless specified.
- ✓ The following sample preparation is designed for the isolation of cells and subsequent total RNA purification from a 30 ml urine sample.

Isolation of Cells from Urine

1. Push up to 30 ml fresh urine[1] with a syringe (not provided) completely through the **ZRC GF™ Filter** to isolate the cells in the filter. Remove urine completely from the filter by pushing through several volumes of air.

   If isolating RNA from microvesicles in urine, do not discard the filtrate (see page 7).

2. Push 700 µl **Urine RNA Buffer** with a syringe (not provided) through the filter and collect the flow-through[2] in a nuclease-free tube (not provided). Push several volumes of air through the filter and collect any residual flow-through. Mix the contents in the tube briefly by vortexing.

3. Proceed to RNA Purification, page 6.

1 Up to 200 ml urine can be processed by repeating the syringe filtration step using the same filter. RNA recovery will be proportional to the amount of urine filtered.
2 The flow-through can be used immediately for RNA purification or can be stored. The RNA in the sample is stable for up to 7 days at room temperature, 2 weeks at 0-8°C, or up to 6 months at -20°C. For long term storage, store at -70°C. Let the sample acclimate to room temperature prior to purifying the RNA.

(III) RNA Purification

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

1. To the flow-through, add 1 volume ethanol (95-100%) (1:1) and mix well.

   Example: Add 700 µl ethanol to 700 µl flow-through.

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

   Optional: At this point, DNase I treatment can be performed (page 7).

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

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

5. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).

6. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

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

   The eluted RNA can be used immediately or stored frozen.

1 To process samples > 700 µl, columns may be reloaded.

Appendices

DNase I Treatment (in-column)

- ✓ Perform DNase I treatment with DNase I Set (#E1010) and RNA Wash Buffer (concentrate; #R1003-3-6); available separately.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Following RNA binding step (page 6, step 2), 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** (see table below) in an RNase-free tube (not provided) and mix by gentle inversion. Then add 40 µl directly into column matrix and incubate at room temperature (20-30°C) for 15 minutes. Proceed with the purification (page 6, step 3).

DNase I Reaction Mix	
DNase I (reconstituted; 1 U/ul)[1]	5 µl
DNA Digestion Buffer	35 µl

Isolation of Microvesicles and Microvesicular RNA from Urine

1. Following the passage of urine through the **ZRC GF™ Filter** (page 5, step 1), save the filtrate!
2. Microvesicles can be isolated by:
   a. Ultracentrifugation (e.g., 118,000 x g for 70 minutes at 4°C; discard the supernatant). Then add 700 µl **Urine RNA Buffer** to resuspend the pellet and mix well.
   b. Filtration Method (e.g., Amicon filter unit, Millipore or similar). Then elute the filter containing the isolated microvesicles with 700 µl **Urine RNA Buffer** and mix well.
3. Proceed to the RNA Purification protocol, page 6, step 1.

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1 Prior to use, reconstitute the lyophilized **DNase I** with 275 µl DNase/RNase-Free Water. Mix by gentle inversion and 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/ml of reaction mixture at 25°C.

Ordering Information

Product Description	Catalog No.	Size
ZR Urine RNA Isolation Kit™	R1038 R1039	20 preps. 50 preps.

Individual Kit Components	Catalog No.	Amount
Urine RNA Buffer	R1038-2-20 R1038-2-50	20 ml 50 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25	10 ml 25 ml
RNA Wash Buffer (concentrate)	R1003-3-12 R1003-3-24	12 ml 24 ml
DNase/RNase-Free Water	W1001-1 W1001-6	1 ml 6 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
Zymo-Spin™ IC Columns	C1004-50	50
Collection Tubes	C1001-50	50
ZRC GF™ Filters	C1009-20 C1009-50	20 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 RNA from any sample:

Quick-RNA kits	
Miniprep Plus #R1057/R1058	≤ 10^7 cells, ≤ 50 mg tissue
MagBeads #R2132/R2133	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

Notes

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