ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR Urine RNA Isolation Kit™

Catalog Nos. **R1038 & R1039**

Highlights

- Quick, simple and reliable recovery of RNA from cells and biological sediment in urine.
- Ideal for recovering total RNA from large volume liquid samples. Also suitable for isolation of RNA from microvesicles.
- *Fast-Spin* column technology allows RNA to be eluted into minimal volumes (≥ 6 µl).
- Omits the use of organic denaturants as well as proteinases.

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

Product Contents

ZR Urine RNA Isolation Kit™ (Kit Size)	R1038 (20 Preps.)	R1039 (50 Preps.)	Storage Temperature
Urine RNA Buffer	20 ml	50 ml	Room Temp.
RNA Prep Buffer	10 ml	25 ml	Room Temp.
RNA Wash Buffer[1] (concentrate)	12 ml	24 ml	Room Temp.
DNase/RNase-Free Water	1 ml	1 ml	Room Temp.
ZRC GF™ Filter	20	50	Room Temp.
Zymo-Spin™ IC Columns	20	50	Room Temp.
Collection Tubes	20	50	Room Temp.
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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.

Specifications

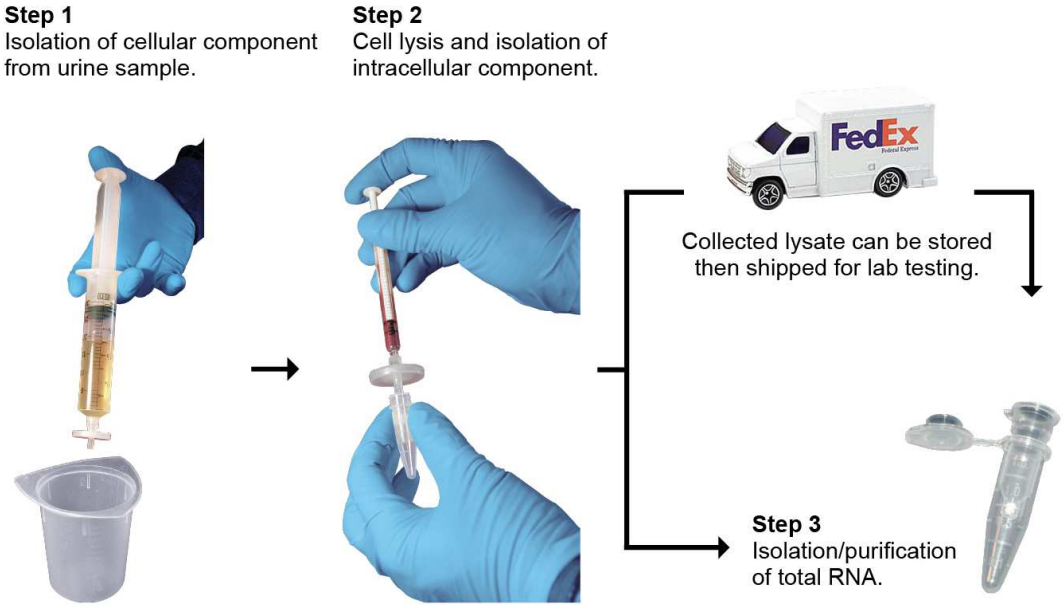
- **Sample Sources** – Urine and other aqueous samples containing cells, biological sediment, microvesicle-associated RNA, etc.
- **Sample Size** – 30 ml (standard reaction); can be increased/decreased proportionally.
- **RNA Recovery** – Typically, 0.2 to 3.0 µg RNA per 30 ml urine sample. The RNA binding capacity of the supplied columns is >5 µg.
- **RNA Purity** – High quality RNA is recovered in RNase-free water. Some DNA may be present in the final preparation of RNA. DNA can be removed by using the **DNA-Free RNA Kit™** (R1013). For *in-column* DNase digestion, see **Appendix** (page 5).
- **RNA Storage** – RNA is eluted with RNase-free water and can be stored at ≤-70 ºC. The addition of RNase inhibitors is recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Note:

[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 of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1039) before use.

Product Description

ZR Urine RNA Isolation Kit™ is an innovative product designed for the easy, reliable and rapid isolation of total RNA from cells in urine samples. The product enables isolation of cells from urine using a syringe and a uniquely-designed syringe filter. Following separation, cells are lysed and total RNA 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, the Urine RNA Buffer 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**. The RNA isolation procedure is simple and can be performed in less than 5 minutes. Use of the ZR Urine RNA Isolation Kit™ results in the isolation of high-quality, total RNA from urine samples that is suitable for subsequent analyses of gene expression that include RT-PCR.



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

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 (R1038) or 96 ml 100% ethanol (104 ml of 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1039).

Protocol

Isolation of Cells from Urine

The following protocol is designed for the isolation of cells and subsequent purification of RNA from a 30 ml sample of urine[1].

1. Take up 30 ml fresh urine in a 30 ml syringe[1]. Push the urine completely through the provided **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 **Appendix II** for instructions (p. 5).

2. Using a 1 ml syringe, push 700 µl **Urine RNA Buffer** through the filter and collect the flow-through[2] in an RNase-free 1.5 ml tube. Push several volumes of air through the filter and collect any residual flow-through. Mix the contents in the tube briefly by vortexing.

Isolation of RNA

3. Add 1 volume (700 µl) ethanol (95-100%) to the tube containing the flow-through and mix briefly[3].

4. Transfer the mixture to a **Zymo-Spin™ IC Column** in a **Collection Tube**. Centrifuge at ≥12,000 x *g* for 1 minute. Discard the flow-through.

   At this point, RNA samples can be DNase treated. See **Appendix I** for instructions (p. 5).

5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge at ≥12,000 x *g* for 1 minute. Discard the flow-through.

6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow-through. Repeat the wash step with 400 µl **RNA Wash Buffer**.

7. Centrifuge the **Zymo-Spin™ IC Column** in an emptied **Collection Tube** at ≥12,000 x *g* for 2 minutes. Remove the **Zymo-Spin™ IC Column** carefully from the **Collection Tube** and transfer it into an RNase-Free Tube.

8. Add ≥6 µl **DNase/RNase-Free Water**[4] directly to the column matrix. Wait for 1 minute then centrifuge at ≥10,000 x *g* for 1 minute and collect the eluted RNA. The RNA can be used immediately or stored at -70 °C.

Notes:

[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.

[3] Maximum loading volume for **Zymo-Spin™ IC** is 800 µl. Column has to be reloaded to process volumes >800 µl.

[4] To maximize RNA yield, increase the elution volume (15 µl recommended) and/or repeat the elution.

Ordering Information

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

For Individual Sale	Catalog No.	Amount
Urine RNA Buffer	R1038-2-20 R1038-2-50	20 ml 50 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
RNA Prep Buffer	R1060-2-10 R1060-2-25	10 ml 25 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
ZRC GF™ Filter	C1009-20 C1009-50	20 50

Appendix I

RNA purification with *in-column* DNase digestion[1]

1. Following *Step 1-4* in the Protocol, make 30 µl **DNase I** cocktail for each sample to be treated:

Example:	RNase-Free DNase I	3 µl (1 U/µl)[1]
	10x Reaction Buffer	3 µl
	RNA Wash Buffer[2]	24 µl

2. Add 400 µl **RNA Wash Buffer** to the **Zymo-Spin™ IC Column** in a **Collection Tube** and centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow through.
3. Add 30 µl DNase I cocktail from *Step 1* directly to the matrix of the **Zymo-Spin™ IC Column**. Keep the **Zymo-Spin™ IC Column** in the **Collection Tube**.
4. Incubate the column at 25-37 ºC for ≥15 minutes[3], then centrifuge at ≥12,000 x *g* for 30 seconds. Discard the flow-through.

   Continue with *Step 5* in the standard Protocol.

Appendix II

Isolation of Microvesicles and Microvesicular RNA from Urine

1. Following the passage of urine through the **ZRC GF™ Filter** (*Step* 1 in the protocol, page 3), **save the filtrate**.
2. Microvesicles can be isolated either by **ultracentrifugation** (e.g., 118,000 x *g* for 70 minutes at 4 °C; discard the supernatant) or **filtration** method (*e.g.,* Amicon filter unit, Millpore or similar).
3. Add 700 µl **Urine RNA Buffer** to resuspend the pellet after ultracentrifugation or elute the filter containing the isolated microvesicles with 700 µl **Urine RNA Buffer**. Mix well. Proceed to **Isolation of RNA** (*Step* 3, page 3).

Notes:

[1] The DNase digestion procedure can be performed using any source of RNase-free DNase I together with its 10X reaction buffer (*e.g.,* 100 U **RNase-free DNase I** (1 U/µl) w/ 10x Reaction Buffer – Zymo Research Catalog - **E1007**).

For *in-tube* DNase treatment and RNA clean-up see the **DNA-Free RNA Kit** (R1013) or the **RNA Clean & Concentrator**™ (R1015, R1017).

To treat 1 µg RNA sample with DNase I, use of 1 unit enzyme is recommended.

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] DNase I maintains activity in the **RNA Wash Buffer** provided in this kit.

[3] The temperature optimum for DNase I activity is at 37 ºC. An optimal incubation time may vary.

Related Products

Product	Description	Prep/Format	Catalog
	Total RNA Purification		
ZR Whole-Blood RNA MiniPrep™	whole blood, partitioned blood	50/column 100/column	**R1020** **R1021**
ZR-96 Whole-Blood RNA Kit™		2x96/plate	**R1022**
ZR Viral RNA Kit™	plasma, serum, liquids, cells, tissue	50/column 200/column	**R1034** **R1035**
ZR-96 Viral RNA Kit™		2x96/plate 4x96/plate	**R1040** **R1041**
ZR Urine RNA Isolation Kit™	urine, liquid samples	50/column	**R1039**
***Quick-RNA*™ MicroPrep**	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids	50/column	**R1050**
***Quick-RNA*™ MiniPrep**		50/column 200/column	**R1054** **R1055**
***Quick-RNA*™ MidiPrep**		25/column	**R1056**
ZR-96 *Quick-RNA*™		2x96/plate 4x96/plate	**R1052** **R1053**
ZR RNA MicroPrep™	cells, tissue, buccal cells, buffy coat, plasma, serum, biological liquids; DNA removal column, small-RNA recovery (≥17nt), *in-column* DNase treatment protocol	50/column 200/column	**R1060** **R1061**
ZR RNA MiniPrep™		50/column 200/column	**R1064** **R1065**
Pinpoint™ Slide RNA Isolation System Kit I	fresh tissue sections	50/column	**R1003**
Pinpoint™ Slide RNA Isolation System Kit II	paraffin-embedded tissue	50/column	**R1007**
ZR Fungal/Bacterial RNA MicroPrep™	bacteria, yeast, fungi; BashingBead™ lysis	50/column	**R2010**
ZR Fungal/Bacterial RNA MiniPrep™		50/column	**R2014**
ZR Plant RNA MiniPrep™	plant parts and tissues; BashingBead™ lysis, RT/PCR inhibitor removal	50/column	**R2024**
ZR Tissue & Insect RNA MicroPrep™	insect, small tissue samples; BashingBead™ lysis	50/column	**R2030**
YeaStar RNA Kit™	yeast, fungi	40/column	**R1002**
	RNA Clean-up, Concentration & Recovery		
RNA Clean & Concentrator™-5	modified/labeled/impure/diluted RNA; small-RNA recovery (≥17nt); *acid phenol* extracted RNA directly from aqueous phase, *in-column* DNase treatment protocol	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™		2x96/plate	**R1080**
DNA-Free RNA Kit™	DNase I treatment; small-RNA recovery (≥17nt)	50/column 200/column	**R1013** **R1014**
Zymoclean™ Gel RNA Recovery Kit	agarose gel separated RNA	50/column	**R1011**
ZR small-RNA™ PAGE Recovery Kit	polyacrylamide gel separated RNA; small-RNA recovery (≥17nt)	20/column	**R1070**
	DNA/RNA Parallel Purification		
ZR-*Duet*™ DNA/RNA MiniPrep	cells, tissue, liquids; DNA/RNA separation, small-RNA recovery (≥17nt), *in-column* DNase treatment protocol	50/column	**D7001**
	DNA/RNA Co-Purification		
ZR Viral DNA/RNA Kit	plasma, serum, liquids, cells, tissue	25/column 100/columns	**D7020** **D7021**