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 total RNA from cells, biological sediment in urine, large volume liquid samples and suitable for isolation of RNA from microvescicles.
- Clean-Spin™ column technology allows RNA to be eluted into volumes ≥6 µl and is ready for use in RT-PCR and other RNA-based procedures.

Contents

Product Contents 1
Specifications.....1
Product Description.....2
Buffer Preparation.....3
Protocol.....3
Ordering Information 4
Appendices 5
Related Products 6

ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

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

Note:

[1] Before use, 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).

Product Contents

ZR Urine RNA Isolation Kit™ (Kit Size)	**R1038** (20 Preps.)	**R1039** (50 Preps.)
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

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.

Specifications

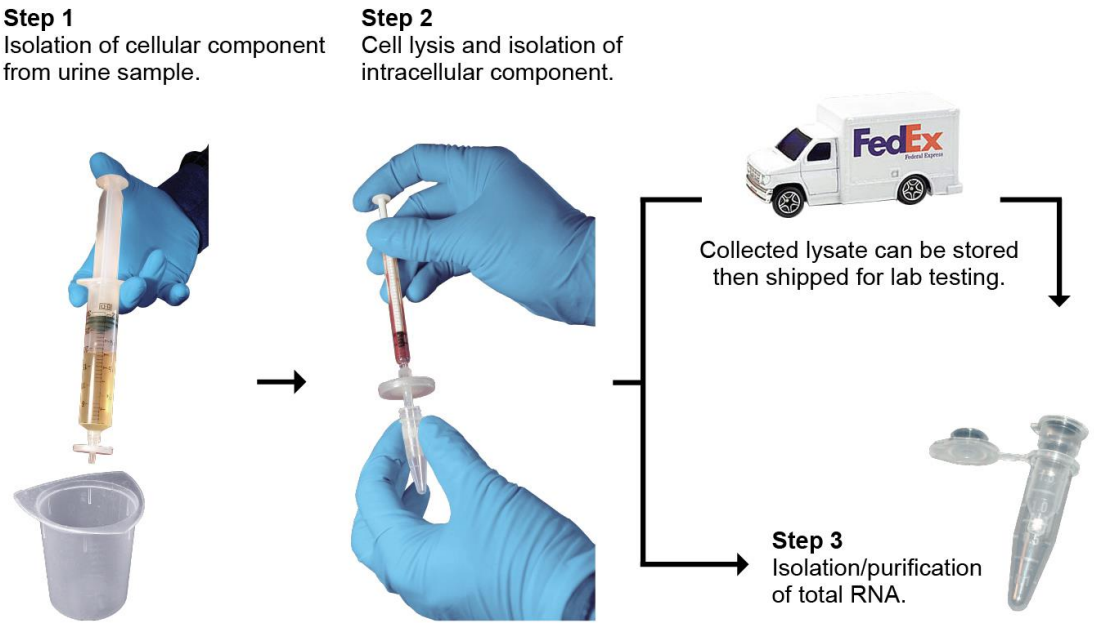
- **Sample Sources** – Urine and other aqueous samples containing cells, biological sediment, microvesicle-associated RNA, etc.
- **Sample Size** – 30 ml (standard reaction); can be increased/decreased proportionally.
- **RNA Recovery** – Typically, 0.2 to 3.0 µg RNA per 30 ml urine sample. The RNA binding capacity of the supplied columns is up to 10 µg.
- **RNA Purity** – High quality RNA is recovered in RNase-free water. Some DNA may be present in the final preparation of RNA. Complete removal of DNA can be accomplished by performing an in-column DNase I digestion (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; syringes (i.e., 30 ml, 1 ml).

 This product is for research use only and should only be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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 (not provided) 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.



*Syringes not provided

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. Push up to 30 ml fresh urine 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** (page 5).

2. Push 700 µl **Urine RNA Buffer** with a syringe (not provided) through the filter and collect the flow-through[2] in an RNase-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.

RNA Purification

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

3. Add 1 volume (700 µl) ethanol (95-100%) to the tube containing the flow-through and mix briefly.
4. Transfer the mixture to a **Zymo-Spin™ IC Column**[3] in a **Collection Tube** and centrifuge for 30 seconds[4]. Discard the flow-through.
5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).
8. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

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

   The eluted 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 ºC, or up to 6 months at -20 ºC. For long term storage, store at -70 ºC. Let the sample acclimate to room temperature prior to purifying the RNA.

[3] To process samples >800 µl, reload the column and repeat or use a vacuum manifold.

[4] At this point, RNA samples can be in-column DNase I treated (page 5).

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

Notes:

[1] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. 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/min/ml of reaction mixture at 25°C.

In-Column **DNase I Digestion**

The DNase I digestion procedure can be performed using **DNase I Set** (E1010)[1].
All centrifugation steps should be performed at 10,000-16,000 *x g.*

1. Following the RNA binding step (page 3, step 4), prewash the column with 400 µl **RNA Wash Buffer**. Centrifuge for 30 seconds. Discard the flow-through.

2. For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	35 µl

3. Add 40 µl of the **DNase I Reaction Mix** directly to the column matrix. Incubate the column at room temperature (20-30°C) for 15 minutes. Then continue with Protocol (RNA Purification, page 3, step 5).

Isolation of Microvesicles and Microvesicular RNA from Urine

1. Following the passage of urine through the **ZRC GF™ Filter** (page 3, 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.

2. Continue with Protocol (RNA Purification, page 3, step 3).

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**