ZYMO RESEARCH

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INSTRUCTION MANUAL

*Quick-cf*RNA™ Serum & Plasma Kit

Catalog No. **R1059**

Highlights

- High-quality cell-free RNA is easily and robustly purified from up to 3 ml of plasma, serum, or other biological fluids.
- Purified cell-free RNA is immediately ready for downstream applications, including RT-qPCR and Next-Generation Sequencing.
- Up to 515x more microRNA is recovered compared to comparable products, enabled by innovative binding system.

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

Product Contents

***Quick-cf*RNA™ Serum & Plasma Kit** (Kit Size)	**R1059** (50 Preps.)	**Storage Temperature**
Proteinase K & Storage Buffer	3 x 20 mg	-20 ºC (after mixing)
***Quick-cf*RNA™ Digestion Buffer**	50 ml	Room Temp.
***Quick-cf*RNA™ Binding Buffer**	100 ml	Room Temp.
RNA Recovery Buffer	10 ml	Room Temp.
RNA Prep Buffer	50 ml	Room Temp.
RNA Wash Buffer (Concentrate)	12 ml	Room Temp.
Spin-Away™ Filters	50	Room Temp.
25 ml Reservoirs	50	Room Temp.
Zymo-Spin™ IC Columns	50	Room Temp.
Collection Tubes	100	Room Temp.
DNase/RNase-Free Water	4 ml	Room Temp.
Instruction Manual	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.

Specifications

- **Sample Types** – 1 ml of plasma, serum, cerebrospinal fluids, amniotic fluids, urine, or other biological fluids. Max volume of 3 ml input can be processed per filter (see Appendix C, Page 6).
- **Purity** – High quality cell-free RNA (cfRNA) is ready for all sensitive downstream applications such as RT-qPCR and Next-Generation Sequencing.
- **Size** – RNA ≥ 17 nt.
- **Yield** – Yields are sample dependent and may vary. Typical recovery ranges from 1 – 100 ng/ml of human plasma or serum.[1] Kit is optimized for recovery of total cfRNA, including small RNA and microRNA.
- **Elution volume** – cfRNA can be eluted into 15 µl (to maximize total yield) or as little as 6 µl (for highly concentrated eluate) of **DNase/RNase-Free Water**.
- **Processing time** – Typically requires 30 minutes hands-on time to process 10 samples. Sample digestion requires 2 hours.
- **Required Equipment** – Microcentrifuge, vortex, water bath or heating block, vacuum, and vacuum manifold.

Notes:

[1] Quantified using fluorescence-based technology (Qubit 3.0 from Thermo Fisher Scientific)

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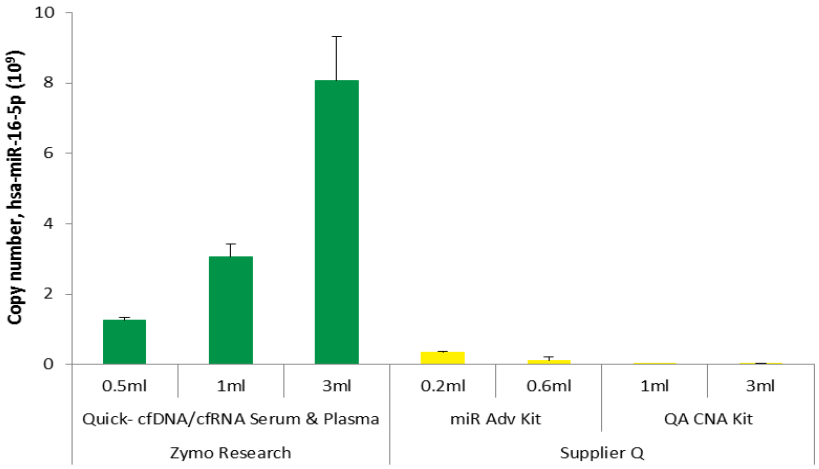
Product Description & Procedure Overview

The ***Quick-cf*RNA™ Serum & Plasma Kit** enables simple and efficient preparation of total cell-free RNA (including protein-bound, exosomal, microRNA, and other small RNA) from serum, plasma, and other biological fluids. The kit does not use toxic phenol-chloroform or cumbersome protein precipitation and removal steps. Recovered cell-free RNA scales linearly relative to the sample input volume up to 3 ml. Zymo-Spin™ Technology enables isolation of ultra-pure cell-free RNA suitable for all subsequent analyses, such as RT-qPCR and NGS.



Isolate up to 515x more microRNA compared to two separate kits from supplier Q. Samples from the same donor (plasma from 61y-F) were processed using the manufacturers' suggested protocol and eluted in 30 µl. Quantification of human *miR-16-5p* was assayed using the method described in Busk P. K., *BMC Bioinformatics*, 2014. The microRNA yield from the *Quick-cf*RNA Serum & Plasma scales linearly to input volume.

Extraction Kit	Zymo	Supplier Q
Reads	1,041,574	905,153
% reads passing filters	76.6%	58.5%
% align to hg19	88.8%	63.9%
% align to miRBase	43.6%	0.4%
miRNAs detected	623	160

Obtain robust cell-free RNA sequencing results. Total cell-free RNA was isolated from 200 µl plasma from four different donors. RealSeq-Biofluids Library Prep Kit (SomaGenics) was used to generate RNA sequencing library and ran on MiniSeq System (Illumina). Averages of reads, read quality, alignments, and microRNA diversity quantification are summarized above. Cell-free RNA isolated using *Quick-cf*RNA™ Serum & Plasma yielded higher quality reads, better alignment to sequence databases, and achieved recovery of 463 more microRNA species.

Notes:

Reagent Preparation

- ✓ Prior to use, reconstitute lyophilized 20 mg **Proteinase K** with 1,040 µl **Proteinase K Storage Buffer**. Vortex to dissolve. Store at -20 ºC.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** (Concentrate).

Protocol

All centrifugation steps should be performed at ≥ 12,000 *x g* for 30 seconds in a microcentrifuge unless specified.
All steps should be performed at room temperature (20-30 ºC).
If processing more than 1 ml per isolation process, please refer to Appendix C for more information.
For rapid preparation, see **Quick Setup Guide** below.

1. Centrifuge samples ≥ 12,000 *x g* for 15 minutes to remove any cell debris and precipitate.[1]

2. In a new 15 ml conical tube, add 200 µl ***Quick-cf*RNA™ Digestion Buffer** per 200 µl of sample (plasma, serum, or other biological fluids) and mix by pipetting. If the input sample volume is ≥ 1.5 ml, use a 50 ml conical tube.

3. Add 10 µl **Proteinase K** per 200 µl of sample and mix thoroughly by vortexing for 10 seconds. Incubate at 37 °C for 2 hours.

4. Add 1 volume ***Quick-cf*RNA™ Binding Buffer** to the digested sample and mix thoroughly by vortexing for 10 seconds.

   Example: Add 400 µl binding buffer to 410 µl digested mixture.

5. Add 1.5 volumes 100% isopropanol to the mixture from **Step 4** and mix thoroughly by vortexing for 10 seconds.

   Example: Add 1.2 ml of 100% isopropanol to 810 µl sample mixture.

Quick Setup Guide

	Sample volume	200 µl	500 µl	1 ml
Step 1	***Quick-cf*RNA™ Digestion Buffer**	200 µl	500 µl	1 ml
Step 2	**Proteinase K**	10 µl	25 µl	50 µl
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours				
Step 3	***Quick-cf*RNA™ Binding Buffer**	400 µl	1 ml	2 ml
Step 4	**100% Isopropanol**	1.2 ml	3 ml	6 ml
	Final mixture volume	2 ml	5 ml	10 ml

6. Insert **25 ml Reservoir** into **Spin-Away™ Filter**. Place the assembly onto vacuum manifold.[2]

[1] Centrifugation is necessary for efficient depletion of residual cells, cell debris, and particulate matter. If cryoprecipitates are visible after freeze-thaw cycles, it is recommended that these are removed by centrifuging again, prior to digestion.

For recommended plasma preparation methods, see Appendix B and references mentioned below:

a) Tuck MK, et al. *Journal of Proteome Research*. 2009 Jan; 8(1):113-117.

b) Köberle V, et al. *PLoS One*. 2013 Sep; 8(9): e75184

c) Peter B. Gahan. *Circulating Nucleic Acids In Early Diagnosis, Prognosis and Treatment Monitoring: An Introduction.* pg. 44-66.

[2] The vacuum pump should be able to apply at least 400 mmHg pressure.

7. Load the entire mixture into the reservoir and turn on the vacuum until the entire mixture has been completely drawn through the Spin-Away™ Filter.[1] Turn off the vacuum pump and discard the reservoir.

8. Add 600 µl **RNA Prep Buffer** and turn on the vacuum until all of the liquid completely passes through the Spin-Away™ Filter. Turn off the vacuum.

9. Transfer the Spin-Away™ Filter to a **Collection Tube** and centrifuge for 2 minutes to remove residual buffer. Place the filter into a new microcentrifuge tube (not provided).

10. Add 200 µl **RNA Recovery Buffer** directly to the Spin-Away™ Filter. Incubate for 3 minutes and centrifuge. **Save the Flow-Through!!!**

11. Add 300 µl ethanol (95-100%) to the flow-through. Mix by pipetting and transfer into a Zymo-Spin™ IC Column in a new Collection Tube. Centrifuge and discard the flow-through.

12. Add 400 µl **RNA Prep Buffer** to the column. Centrifuge and discard the flow-through.

13. Add 700 µl **RNA Wash Buffer** to the column. Centrifuge and discard the flow-through.

14. 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 into a clean microcentrifuge tube (not provided).

15. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix, incubate for 2 minutes and centrifuge to elute cfRNA.[2]

    The eluted cfRNA can be used immediately or stored at -70 °C.[3]

Notes:

[1] For 3 ml of sample volume, it may take up to 15 minutes for the digestion mixture to completely pass through the filter.

[2] Alternatively, for highly concentrated cfRNA, use ≥ 6 µl elution volume.

[3] Quantification of cfRNA requires highly sensitive assays due to very low amounts found in biological fluids. Please see Appendix B for details.

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 A260 units/min/mL of reaction mixture at 25°C.

Appendix A: DNase I Treatment (Optional)

For complete removal of cfDNA from cfRNA eluate, the DNase digestion procedure can be performed using the **DNase I Set** (Cat. No. E1010)[1] and the **Oligo Clean & Concentrator™** (Cat. No. D4060, D4061).

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

Eluate volume adjusted with water or TE buffer	40 µl
DNase I	5 µl
DNA Digestion Buffer	5 µl
Total Volume	50 µl

Incubate at room temperature (20-30°C) for 15 minutes then start with Step 1 of **Oligo Clean & Concentrator™** protocol.

Appendix B: Sample Handling and Quantification Methods

• Cell-free nucleic acid is protected from the denaturing environment of biofluids by encapsulation in extracellular vesicles or binding to proteins. We recommend minimizing freeze-thaw cycles and to avoid vigorous shaking prior to sample digestion to preserve integrity of the protective vesicles and proteins.

• Slow thawing of biofluids at 4 °C increases the formation of precipitate that is hard to digest and may clog columns. We recommend thawing plasma samples at room temperature or 37 °C to minimize the formation of precipitate. Remove precipitate by centrifuging samples at 12,000 *x g* for 15 minutes at room temperature. Precipitate can cause inefficient digestion, which can lead to filter clogging and lower yield.

• Highly sensitive quantification methods are needed to accurately measure dilute eluates such as cell-free nucleic acid from biological fluids. We recommend using fluorescence-based detection methods (e.g. Qubit from Thermo-Fisher) or sensitive electrophoresis systems (e.g. TapeStation or Bioanalyzer from Agilent).

• If a sample-to-sample variation control is needed in microRNA RT-qPCR analysis, an exogenous microRNA can be used. We recommend spiking 1 to 10 pg of an exogenous microRNA (e.g. *cel-miR-39-3p* microRNA; sequence available at mirbase.org) to each prep after completion of sample digestion (after Step 2).

Appendix C: Processing High Sample Input Volume Up to 3 ml

- The kit is supplied with enough reagents to process up to a max of 1 ml sample input.
- To process all 50 preps with 2 ml sample input, purchase the following additional buffers:
  - 3 of Proteinase K & Storage Buffer, 20 mg set (Catalog No. D-3001-2-20)
  - 1 of *Quick-cf*RNA™ Digestion Buffer, 50 ml (Catalog No. R1059-3-50)
  - 1 of *Quick-cf*RNA™ Binding Buffer, 100 ml (Catalog No. R1059-4-100)
- To process all 50 preps with 3 ml sample input, purchase the following additional buffers:
  - 6 of Proteinase K & Storage Buffer, 20 mg set (Catalog No. D-3001-2-20)
  - 2 of *Quick-cf*RNA™ Digestion Buffer, 50 ml (Catalog No. R1059-3-50)
  - 2 of *Quick-cf*RNA™ Binding Buffer, 100 ml (Catalog No. R1059-4-100)
- For rapid preparation, see **Quick Setup Guide** below.

Quick Setup Guide

	Sample volume	1 ml	2 ml	3 ml
Step 1	***Quick-cf*RNA™ Digestion Buffer**	1 ml	2 ml	3 ml
Step 2	**Proteinase K**	50 µl	100 µl	150 µl
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours				
Step 3	***Quick-cf*RNA™ Binding Buffer**	2 ml	4 ml	6 ml
Step 4	**100% Isopropanol**	6 ml	12 ml	18 ml
	Final mixture volume	10 ml	20 ml	30 ml

- Proceed to Step 6 (Page 3) to continue isolation process.
- Please refer to Ordering Information (Page 8) for ordering details on all kit components.

Troubleshooting:

For Technical Assistance, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com

Problem	Possible Causes and Suggested Solutions
Spin-Away Filter™ Clogging	Some biological fluids, such as plasma, are protein-rich and require complete Proteinase K digestion prior to passing through the Spin-Away Filter™. Removing visible precipitate prior to processing is recommended for optimal kit performance. Although digesting samples for 2 hours at 37 °C is sufficient for most samples, extending the digestion time to 3-4 hours may help if samples contain residual precipitate that is hard to remove. Do not add more than the indicated amount of proteinase K Do not increase the digestion temperature above 37 °C. Increased vacuum pressures (≥ 400 mmHg) can help the lysate flow through the filter faster.
Low Yield	Yield can vary significantly from donor to donor. Increased levels of circulating nucleic acids in the blood reflect pathological processes, including cancers, inflammatory diseases, and trauma (Schwarzenbach H. et al. *Nat. Rev. Cancer*, 2011). Incomplete digestion can cause clogging of the Spin-Away™ Filter and can result in inefficient RNA binding. Ensure sample is completely digested before proceeding to sample binding.

Ordering Information

Product Description	Catalog No.	Kit size
***Quick-cf*RNA™ Serum & Plasma Kit**	R1059	50 preps
***Quick-cf*DNA/*cf*DNA™ Serum & Plasma Kit**	R1072	50 preps
***Quick-cf*DNA™ Serum & Plasma Kit**	D4076	50 preps
Oligo Clean & Concentrator™	D4060 D4061	50 preps 200 preps
DNase I Set	E1010	Set

For Individual Sale	Catalog No.	Amount
Proteinase K & Storage Buffer	D3001-2-5 D3001-2-20	5 mg set 20 mg set
***Quick-cf*RNA™ Digestion Buffer**	R1059-3-50	50 ml
***Quick-cf*RNA™ Binding Buffer**	R1059-4-100	100 ml
RNA Recovery Buffer	R1070-1-10	20 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 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
Spin-Away™ Filters	C1006-50-F	50
25 ml Reservoirs	C1039-25	25
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml