



**ZYMO RESEARCH**

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## **Quick-cfDNA/cfRNA™ Serum & Plasma Kit** Catalog No. R1072

### **Highlights**

- High-quality cell-free DNA and RNA are easily and robustly purified from up to 3 ml of plasma, serum, or other biological fluids.
- Purified cell-free DNA and RNA are 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.

### **Contents**

Product Contents .....	1
Specifications.....	1
Product Description.....	2
Procedure Overview.....	3
Reagent Preparation .....	4
Protocol (Parallel Purification) .....	4-6
Appendices	
A. cfDNA/cfRNA Co-Purification Protocol .....	6-7
B. DNase I Treatment.....	8
C. Sample Handling and Quantification Methods .....	8
D. Processing Samples in DNA/RNA Shield .....	9
E. Compatibility with Streck Blood Tubes .....	10
Troubleshooting .....	11
Ordering Information .....	12

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

<b>Quick-cfDNA/cfRNA™ Serum &amp; Plasma Kit (Kit Size)</b>	<b>R1072 (50 Preps.)</b>	<b>Storage Temperature</b>
<b>Proteinase K &amp; Storage Buffer</b>	8 x 20 mg	-20 °C (after mixing)
<b>Quick-cfDNA/cfRNA™ Digestion Buffer</b>	150 ml	Room Temp.
<b>Quick-cfDNA/cfRNA™ Binding Buffer</b>	2 x 150 ml	Room Temp.
<b>Cell-free Recovery Buffer</b>	20 ml	Room Temp.
<b>RNA Prep Buffer</b>	100 ml	Room Temp.
<b>RNA Wash Buffer (Concentrate)</b>	3 x 12 ml	Room Temp.
<b>Spin-Away™ Filters</b>	50	Room Temp.
<b>25 ml Reservoirs</b>	50	Room Temp.
<b>Zymo-Spin™ IC Columns</b>	100	Room Temp.
<b>Collection Tubes</b>	200	Room Temp.
<b>DNase/RNase-Free Water</b>	10 ml	Room Temp.
<b>Instruction Manual</b>	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** – Up to 3 ml of plasma, serum, cerebrospinal fluids, amniotic fluids, urine, or other biological fluids.
- **Purity** – High quality cell-free DNA (cfDNA) and cell-free RNA (cfRNA) are ready for all sensitive downstream applications such as RT-qPCR and Next-Generation Sequencing.
- **Size** – DNA ≥ 50 bp and RNA ≥ 17 nt.
- **Yield** – Yields are sample dependent and may vary. Typical recovery ranges from 1 – 100 ng/ml of human plasma or serum for both cfDNA and cfRNA.<sup>1</sup> Kit is optimized for recovery of total cfDNA and cfRNA, including small RNA and microRNA.
- **Elution Volume** – cfDNA and 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 (cfDNA/cfRNA co-elution protocol) to 45 minutes (cfDNA/cfRNA separation protocol) 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.

<sup>1</sup> Trademarks of Zymo Research Corporation. This product is for research use only and should 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 safety guidelines and rules enacted by your research institution or facility.

### **Notes:**

<sup>1</sup> Quantified using fluorescence-based technology (Qubit 3.0 from Thermo Fisher Scientific)

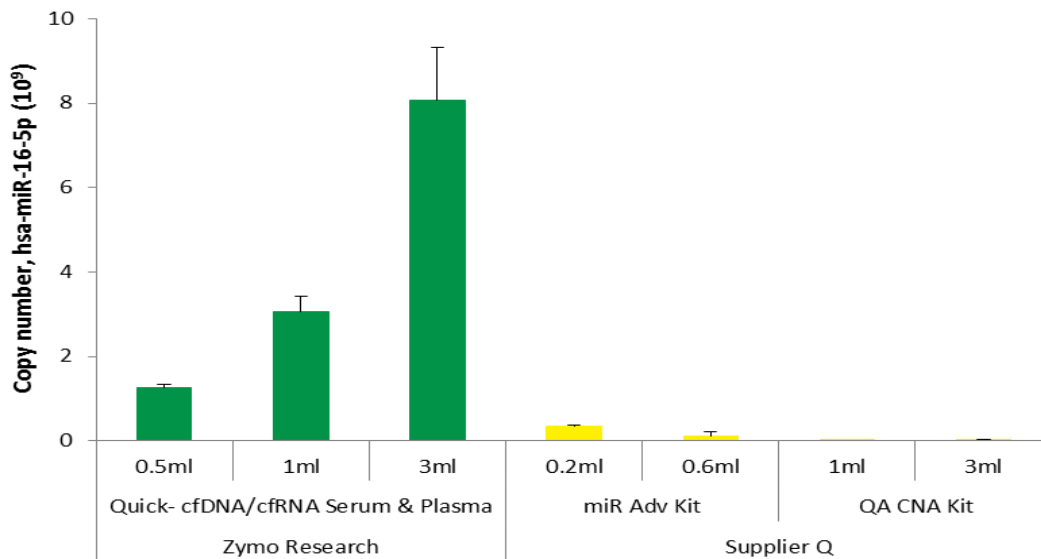
Note – ™ Trademarks of Zymo Research Corporation. This product is for research use only and should be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow safety guidelines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

## **ZYMO RESEARCH CORP.**

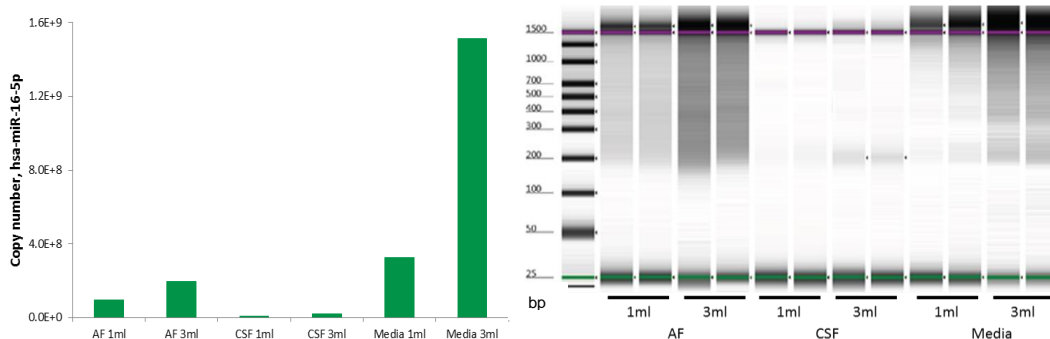
Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ [info@zymoresearch.com](mailto:info@zymoresearch.com) ▪ [www.zymoresearch.com](http://www.zymoresearch.com)

## Product Description

The **Quick-cfDNA/cfRNA™ Serum & Plasma Kit** enables simple and efficient preparation of all circulating cell-free DNA and 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. The kit efficiently separates cell-free DNA and RNA, with option to co-elute. Cell-free nucleic acids scale linearly relative to the sample input volume up to 3 ml. Zymo-Spin™ Technology enables isolation of ultra-pure cell-free nucleic acids 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-cfDNA/cfDNA* Serum & Plasma scales linearly to input volume.

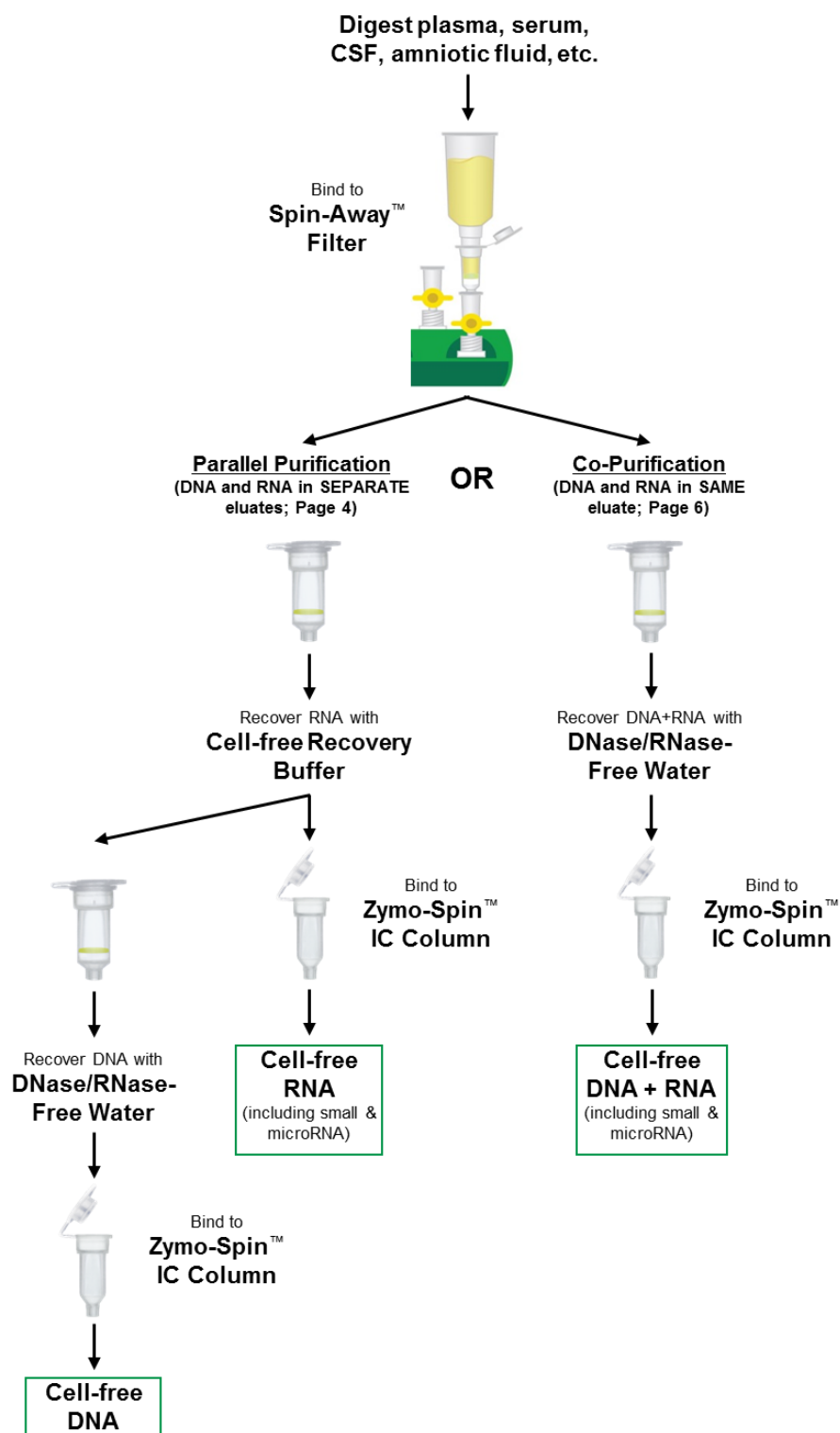


**Proven compatibility with other biological fluid types.** Amniotic fluid (AF), cerebrospinal fluid (CSF), or spent HeLa cell culture media (Media) were used to isolate cell-free nucleic acid. (Left figure) Quantification of human *miR-16-5p* was assayed using the method described in Busk P. K., *BMC Bioinformatics*, 2014. (Right figure) Endogenous cell-free DNA from each sample type visualized using the Agilent Tapestation 2200 (DNA HS ScreenTape).

### ZYMO RESEARCH CORP.

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

## Procedure Overview



**ZYMO RESEARCH CORP.**

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

## 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 °C.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** (Concentrate).

## Protocol: Parallel Purification

All centrifugation steps should be performed at  $\geq 12,000 \times g$  for 30 seconds in a microcentrifuge unless specified. All steps should be performed at room temperature (20-30 °C). For rapid preparation, see **Quick Setup Guide** below.

1. Centrifuge samples  $\geq 12,000 \times g$  for 15 minutes to remove any cell debris and precipitate.<sup>1</sup>
2. In a new 15 ml conical tube, add 200 µl **Quick-cfDNA/cfRNA™ Digestion Buffer** per 200 µl of sample (plasma, serum or other biological fluids) and mix by pipetting. If the input sample volume is  $\geq 1.5$  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-cfDNA/cfRNA™ 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	3 ml (max)
<b>Step 1</b>	<b>Quick-cfDNA/cfRNA™ Digestion Buffer</b>	200 µl	500 µl	1 ml	3 ml
<b>Step 2</b>	<b>Proteinase K</b>	10 µl	25 µl	50 µl	150 µl
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours					
<b>Step 3</b>	<b>Quick-cfDNA/cfRNA™ Binding Buffer</b>	400 µl	1 ml	2 ml	6 ml
<b>Step 4</b>	<b>100% Isopropanol</b>	1.2 ml	3 ml	6 ml	18 ml
	<b>Final mixture volume</b>	2 ml	5 ml	10 ml	30 ml

6. Insert the **25 ml Reservoir** into the **Spin-Away™ Filter** and place the assembly onto the vacuum manifold.<sup>2</sup>

### Notes:

<sup>1</sup> 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 C 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.

<sup>2</sup> The vacuum pump should be able to apply at least 400 mmHg pressure.

### ZYMO RESEARCH CORP.

**Notes:**

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

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.<sup>1</sup> 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 **Cell-free Recovery Buffer** directly to the Spin-Away™ Filter. Incubate for 3 minutes and centrifuge. Save the Flow-Through!

**Flow-Through:**  
**Cell-free RNA Isolation**

- A1. Add 300 µl ethanol (95-100%) to the flow-through. Mix by pipetting.
- A2. Transfer into a **Zymo-Spin™ IC Column** in a new **Collection Tube**.
- A3. Centrifuge and discard the flow-through.
- A4. Continue to **Step 11**.

**Spin-Away™ Filter:**  
**Cell-free DNA Isolation**

- B1. Place the **Spin-Away™ Filter** into a new **Collection Tube**.
- B2. Add 600 µl **RNA Prep Buffer**. Centrifuge and discard the flow-through.
- B3. Add 700 µl **RNA Wash Buffer**. Centrifuge and discard the flow-through. Repeat this step.
- B4. Transfer the Spin-Away™ Filter into a clean microcentrifuge tube (not provided).
- B5. Add 100 µl **DNase/RNase-Free Water** directly to the Spin-Away™ Filter matrix. Incubate for 2 minutes and centrifuge. Discard the filter.
- B6. Add 200 µl **Cell-free Recovery Buffer** to the eluate and mix by pipetting.
- B7. Add 300 µl ethanol (95-100%) and mix by pipetting.
- B8. Transfer the mixture to a **Zymo-Spin™ IC Column** in a new **Collection Tube** and centrifuge. Discard the flow-through.
- B9. Continue to **Step 11**.

11. Add 400 µl **RNA Prep Buffer** to the column. Centrifuge and discard the flow-through.
12. Add 700 µl **RNA Wash Buffer** to the column. Centrifuge and discard the flow-through.

**ZYMO RESEARCH CORP.**

13. 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).

14. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix, incubate for 2 minutes and centrifuge to elute cfDNA/cfRNA.<sup>1</sup>

The eluted cfDNA/cfRNA can be used immediately or stored at -70 °C.<sup>2</sup>

For RNA-seq, we recommend DNase I treatment (refer to Appendix B on page 8).

## Appendix A: cfDNA/cfRNA Co-Purification Protocol

All centrifugation steps should be performed at  $\geq 12,000 \times g$  for 30 seconds in a microcentrifuge unless specified.

All steps should be performed at room temperature (20-30 °C).

For rapid preparation, see **Quick Setup Guide** below.

1. Centrifuge samples  $\geq 12,000 \times g$  for 15 minutes to remove any cell debris and precipitate.<sup>3</sup>

2. In a new 15 ml conical tube, add 200 µl **Quick-cfDNA/cfRNA™ Digestion Buffer** per 200 µl of sample (plasma, serum, or other biological fluids) and mix by pipetting. If the input sample volume is  $\geq 1.5$  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-cfDNA/cfRNA™ 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 volume 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	3 ml (max)
<b>Step 1</b>	<b>Quick-cfDNA/cfRNA™ Digestion Buffer</b>	200 µl	500 µl	1 ml	3 ml
<b>Step 2</b>	<b>Proteinase K</b>	10 µl	25 µl	50 µl	150 µl
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours					
<b>Step 3</b>	<b>Quick-cfDNA/cfRNA™ Binding Buffer</b>	400 µl	1 ml	2 ml	6 ml
<b>Step 4</b>	<b>100% Isopropanol</b>	1.2 ml	3 ml	6 ml	18 ml
	<b>Final mixture volume</b>	2 ml	5 ml	10 ml	30 ml

#### Notes:

<sup>1</sup> Alternatively, for highly concentrated cfDNA/cfRNA, use  $\geq 6$  µl elution volume.

<sup>2</sup> Quantification of cfDNA and cfRNA requires highly sensitive assays due to very low amounts found in biological fluids. Please see Appendix C for details.

<sup>3</sup> Centrifugation is necessary for efficient depletion of residual cells, cell debris, and particulate matter. If cryoprecipitate 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 C 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.

#### ZYMO RESEARCH CORP.

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



**Notes:**

<sup>1</sup> The vacuum pump should be able to apply at least 400 mmHg pressure.

<sup>2</sup> Alternatively, for highly concentrated cfDNA/cfRNA, use  $\geq 6$   $\mu$ l elution volume.

<sup>3</sup> Quantification of cfDNA and cfRNA requires highly sensitive assays due to very low amounts found in biological fluids. Please see Appendix C for details.

6. Insert the **25 ml Reservoir** into the **Spin-Away™ Filter** and place the assembly onto the vacuum manifold.<sup>1</sup>
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. Turn off the vacuum pump and discard the reservoir.
8. Add 600  $\mu$ l **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 100  $\mu$ l **DNase/RNase-Free Water** directly to the Spin-Away™ Filter. Incubate for 2 minutes and centrifuge. Discard the filter.
11. Add 200  $\mu$ l **Cell-free Recovery Buffer** to the eluate and mix by pipetting.
12. Add 450  $\mu$ l ethanol (95-100%) and mix by pipetting.
13. Transfer the mixture to a **Zymo-Spin™ IC Column** in a new **Collection Tube** and centrifuge. Discard the flow-through.
14. Add 400  $\mu$ l **RNA Prep Buffer** to the column. Centrifuge and discard the flow-through.
15. Add 700  $\mu$ l **RNA Wash Buffer** to the column. Centrifuge and discard the flow-through.
16. Add 400  $\mu$ l **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).
17. Add 15  $\mu$ l **DNase/RNase-Free Water** directly to the column matrix, incubate for 2 minutes and centrifuge to elute total cell-free nucleic acid.<sup>2</sup>

The eluted total cell-free nucleic acid can be used immediately or stored at -70 °C.<sup>3</sup> For RNA-seq, we recommend DNase I treatment (refer to Appendix B on page 8).



## **Appendix B: DNase I Treatment**

For complete removal of cfDNA from cfRNA eluate, the DNase digestion procedure can be performed using the **DNase I Set** (Cat. No. E1010)<sup>1</sup> 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 .....	<u>5 µl</u>
<b>Total Volume</b> .....	50 µl

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

### **Notes:**

<sup>1</sup> 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 C: 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 D: Processing Samples in DNA/RNA Shield**

For cell-free biofluid samples stored in DNA/RNA Shield (R1200) at 1:1 ratio, perform the following modified Proteinase K digestion. For rapid preparation, please see **Quick Setup Guide** below.

1. Add 25  $\mu$ l **Proteinase K** per 1 ml samples in DNA/RNA Shield and mix thoroughly by vortexing for 10 seconds. Incubate at 37 °C for 2 hours.  
Example: 500  $\mu$ l biofluid, 500  $\mu$ l DNA/RNA Shield (2X concentrate), 25  $\mu$ l Proteinase K.
2. Add 1 volume **Quick-cfDNA/cfRNA™ Digestion Buffer** to the digested sample and mix thoroughly by vortexing for 10 seconds.  
Example: Add 1 ml *Quick-cfDNA/cfRNA™* Digestion Buffer to 1 ml digested mixture.
3. Add 1 volume **Quick-cfDNA/cfRNA™ Binding Buffer** to the mixture from **Step 2** and mix thoroughly by vortexing for 10 seconds.  
Example: Add 2 ml *Quick-cfDNA/cfRNA™* Binding Buffer to 2 ml sample mixture.
4. Add 1.5 volume 100% isopropanol to the mixture from **Step 3** and mix thoroughly by vortexing for 10 seconds.  
Example: Add 6 ml of 100% isopropanol to 4 ml sample mixture.

### **Quick Setup Guide**

	Sample/Shield Mixture Volume	200 $\mu$ l	500 $\mu$ l	1 ml	3 ml (max)
<b>Step 1</b>	<b>Proteinase K</b>	5 $\mu$ l	12.5 $\mu$ l	25 $\mu$ l	75 $\mu$ l
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours					
<b>Step 2</b>	<b>Quick-cfDNA/cfRNA™ Digestion Buffer</b>	200 $\mu$ l	500 $\mu$ l	1 ml	3 ml
<b>Step 3</b>	<b>Quick-cfDNA/cfRNA™ Binding Buffer</b>	400 $\mu$ l	1 ml	2 ml	6 ml
<b>Step 4</b>	<b>100% Isopropanol</b>	1.2 ml	3 ml	6 ml	18 ml
	<b>Final mixture volume</b>	2 ml	5 ml	10 ml	30 ml

- Proceed to **Step 6** of Parallel Purification (Page 4) or Co-Purification (Page 7).

## **Appendix E: Compatibility with Streck Blood Tubes**

Plasma/serum samples that are derived from blood stored in Streck blood tubes contain chemically fixed proteins. In order to completely digest samples derived from either Cell-Free DNA BCT® or Cell-Free RNA BCT® from Streck, plasma/serum samples must be incubated in *Quick-cfDNA/cfRNA*™ Digestion Buffer at 55 °C, prior to proteinase K digestion. Please follow the protocol below for isolation of cell-free DNA and RNA from plasma/serum samples derived from Streck Blood Tubes.

1. Centrifuge samples  $\geq 12,000 \times g$  for 15 minutes to remove any cell debris and precipitate.
2. In a new 15 ml conical tube, add 200  $\mu$ l ***Quick-cfDNA/cfRNA*™ Digestion Buffer** per 200  $\mu$ l of sample (plasma, serum, or other biological fluids) and mix by pipetting. If the input sample volume is  $\geq 1.5$  ml, use a 50 ml conical tube.
3. Incubate the mixture at 55 °C for 30 minutes.
  - Proceed to **Step 3** of Parallel Purification (Page 4) or Co-Purification (Page 6).

**Troubleshooting:**

For Technical Assistance, please contact 1-888-882-9682 or E-mail [tech@zymoresearch.com](mailto:tech@zymoresearch.com)

Problem	Possible Causes and Suggested Solutions
<b>Spin-Away Filter™ Clogging</b>	<p>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.</p> <p>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.</p> <p>Do not add more than the indicated amount of proteinase K</p> <p>Do not increase the digestion temperature above 37 °C.</p> <p>Increased vacuum pressures (<math>\geq 400</math> mmHg) can help the lysate flow through the filter faster.</p>
<b>Low Yield</b>	<p>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. <i>Nat. Rev. Cancer</i>, 2011).</p> <p>Incomplete digestion can cause the Spin-Away™ Filter to clog and can result in inefficient nucleic acid binding. Ensure sample is completely digested before proceeding to sample binding.</p>
<b>cfDNA/cfRNA Separation Efficiency</b>	<p>Plasma contains cfDNA and cfRNA bound to protective proteins or inside extracellular vesicles (e.g. exosomes).</p> <p>Majority of cfDNA from healthy individuals' plasma is &lt; 200 bp, double-stranded, and originates from apoptotic white blood cells (Snyder et al. <i>Cell</i>, 2014). Significant portion of cfRNAs are bound and protected by microRNA-processing proteins (Arroyo J. D. et al, <i>PNAS</i>, 2010). Nucleic acid from human plasma derived exosomes is mostly comprised of microRNA and partly of other types of DNA and RNA (Huang et al. <i>BMC Genomics</i>, 2013).</p> <p>cfDNA and cfRNA separation is highly efficient above 50 bp/nt or longer. DNA below 50 bp will spill into cfRNA eluate. However, co-eluting cfDNA and cfRNA does not have negative impact in most downstream applications. In addition, length of most cfDNA is around 160-200 bp and most cfRNA is below 50 nt, which this kit will efficiently separate.</p>

**ZYMO RESEARCH CORP.**

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

**Ordering Information**

Product Description	Catalog No.	Kit size
<b>Quick-cfDNA/cfRNA™ Serum &amp; Plasma Kit</b>	R1072	50 preps
<b>Quick-cfRNA™ Serum &amp; Plasma Kit</b>	R1059	50 preps
<b>Quick-cfDNA™ Serum &amp; Plasma Kit</b>	D4076	50 preps
<b>Oligo Clean &amp; Concentrator™</b>	D4060	50 preps
	D4061	200 preps
<b>DNase I Set</b>	E1010	Set
<b>DNA/RNA Shield™, 2X concentrate</b>	R1200-25	25 ml
	R1200-125	125 ml

For Individual Sale	Catalog No.	Amount
<b>Proteinase K &amp; Storage Buffer</b>	D3001-2-5	5 mg set
	D3001-2-20	20 mg set
<b>Quick-cfDNA/cfRNA™ Digestion Buffer</b>	R1072-1-150	150 ml
<b>Quick-cfDNA/cfRNA™ Binding Buffer</b>	R1072-2-150	150 ml
<b>Cell-free Recovery Buffer</b>	R1072-3-20	20 ml
<b>RNA Prep Buffer</b>	R1060-2-10	10 ml
	R1060-2-25	25 ml
	R1060-2-100	100 ml
<b>RNA Wash Buffer</b> (concentrate)	R1003-3-6	6 ml
	R1003-3-12	12 ml
	R1003-3-24	24 ml
	R1003-3-48	48 ml
<b>Spin-Away™ Filters</b>	C1006-50-F	50
<b>25 ml Reservoirs</b>	C1039-25	25
<b>Zymo-Spin™ IC Columns</b>	C1004-50	50
	C1004-250	250
<b>Collection Tubes</b>	C1001-50	50
	C1001-500	500
	C1001-1000	1000
<b>DNase/RNase-Free Water</b>	W1001-1	1 ml
	W1001-4	4 ml
	W1001-6	6 ml
	W1001-10	10 ml
	W1001-30	30 ml

**ZYMO RESEARCH CORP.**

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