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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.

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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.

Quick-cfDNA/cfRNA™ Serum & Plasma Kit (Kit Size)	R1072 (50 Preps.)	Storage Temperature
Proteinase K & Storage Buffer	8 x 20 mg	-20 °C (after mixing)
Quick-cfDNA/cfRNA™ Digestion Buffer	150 ml	Room Temp.
Quick-cfDNA/cfRNA™ Binding Buffer	2 x 150 ml	Room Temp.
Cell-free Recovery Buffer	20 ml	Room Temp.
RNA Prep Buffer	100 ml	Room Temp.
RNA Wash Buffer (Concentrate)	3 x 12 ml	Room Temp.
Spin-Away™ Filters	50	Room Temp.
25 ml Reservoirs	2 x 25	Room Temp.
Zymo-Spin™ IC Columns	2 x 50	Room Temp.
Collection Tubes	4 x 50	Room Temp.
DNase/RNase-Free Water	10 ml	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 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 \geq 50 bp and RNA \geq 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.¹ 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 μ l (to maximize total yield) or as little as 6 μ l (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.

Notes:

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

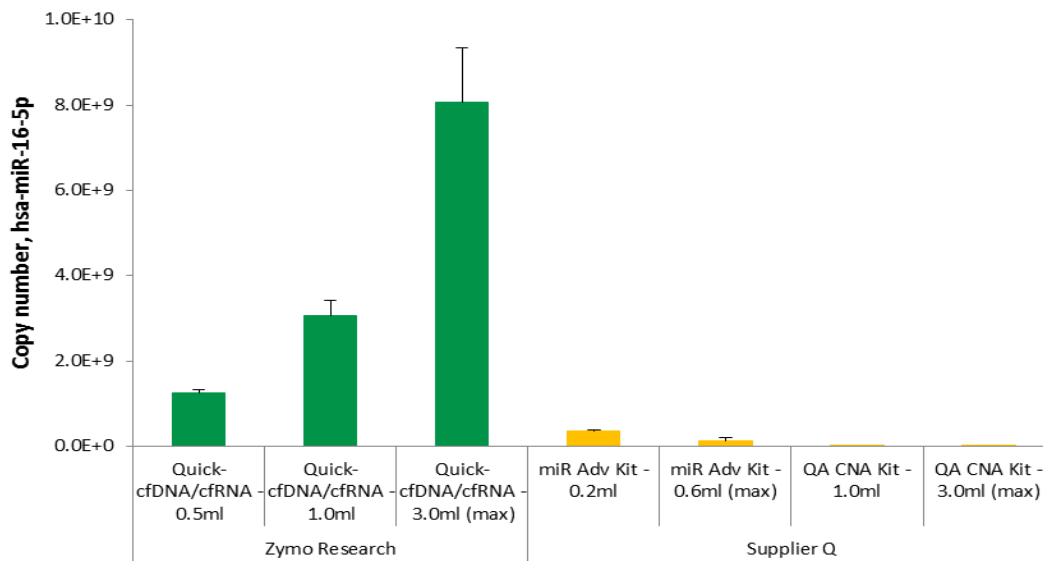
Note – ™ Trademarks of Zymo Research Corporation. This product is for research use only and should be used by trained professionals. It is not intended for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow safety guidelines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

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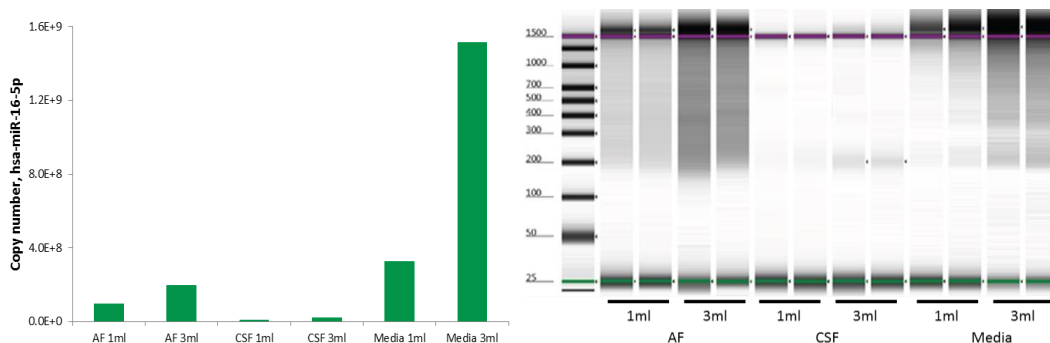
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Product Description

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.



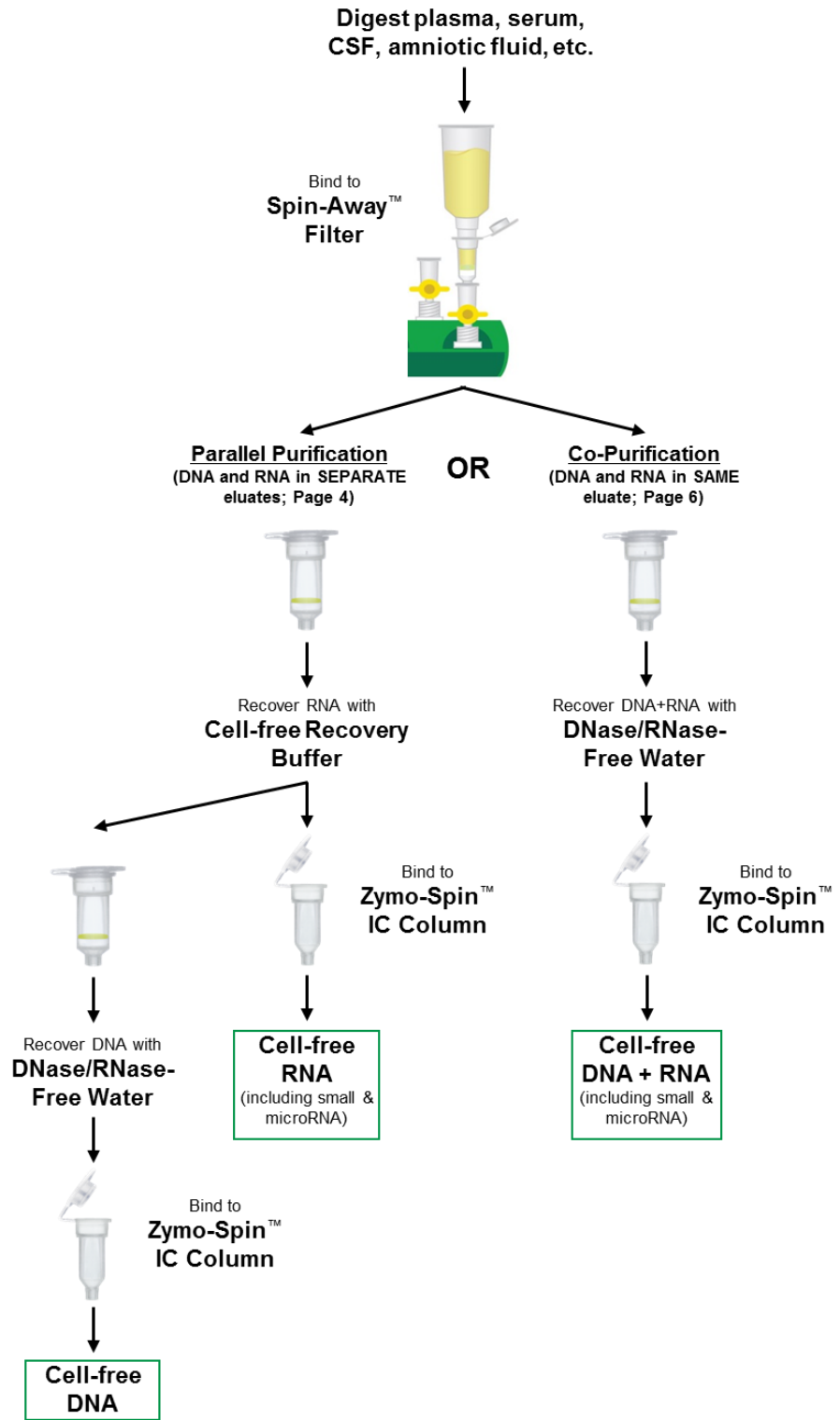
Isolate up to 515x more microRNA compared to two separate kits from supplier Q. Samples from the same donor (plasma from 61y-F) were processed using the manufacturers' suggested protocol and eluted in 30 μ l. Quantification of human *miR-16-5p* was assayed using the method described in Busk P. K., *BMC Bioinformatics*, 2014. Yield 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).

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Procedure Overview



Reagent Preparation

- ✓ Prior to use, reconstitute lyophilized 20 mg **Proteinase K** with 1,040 μ l **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.¹
2. In a new 15 ml conical tube, add 200 μ l **Quick-cfDNA/cfRNA™ Digestion Buffer** per 200 μ l 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 μ l **Proteinase K** per 200 μ l 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 μ l binding buffer to 410 μ l 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 μ l sample mixture.

Quick Setup Guide

	Sample volume	200 μ l	500 μ l	1 ml	3 ml (max)
Step 1	Quick-cfDNA/cfRNA™ Digestion Buffer	200 μ l	500 μ l	1 ml	3 ml
Step 2	Proteinase K	10 μ l	25 μ l	50 μ l	150 μ l
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours					
Step 3	Quick-cfDNA/cfRNA™ Binding Buffer	400 μ l	1 ml	2 ml	6 ml
Step 4	100% Isopropanol	1.2 ml	3 ml	6 ml	18 ml
	Final mixture volume	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.²

Notes:

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 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.

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

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.

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 **Cell-free Recovery Buffer** directly to the Spin-Away™ Filter. Incubate for 3 minutes and centrifuge. **Save the Flow-Through for cfRNA and the Spin-Away™ Filter for cfDNA!!!**

Flow-Through:
cfRNA Isolation

- A1. 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.
- A2. Continue to **Step 11**.

Spin-Away™ Filter: cfDNA 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.

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13. Add 400 μ 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).
14. Add 15 μ l **DNase/RNase-Free Water** directly to the column matrix, incubate for 2 minutes and centrifuge to elute cfDNA/cfRNA.¹

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

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.³
2. In a new 15 ml conical tube, add 200 μ l **Quick-cfDNA/cfRNA™ Digestion Buffer** per 200 μ l 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 μ l **Proteinase K** per 200 μ l 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 μ l binding buffer to 410 μ l 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 μ l sample mixture.

Quick Setup Guide

	Sample volume	200 μ l	500 μ l	1 ml	3 ml (max)
Step 1	Quick-cfDNA/cfRNA™ Digestion Buffer	200 μ l	500 μ l	1 ml	3 ml
Step 2	Proteinase K	10 μ l	25 μ l	50 μ l	150 μ l
Mix thoroughly by vortexing and incubate at 37 °C for 2 hours					
Step 3	Quick-cfDNA/cfRNA™ Binding Buffer	400 μ l	1 ml	2 ml	6 ml
Step 4	100% Isopropanol	1.2 ml	3 ml	6 ml	18 ml
	Final mixture volume	2 ml	5 ml	10 ml	30 ml

Notes:

¹ Alternatively, for highly concentrated cfDNA/cfRNA, use $\geq 6 \mu$ l elution volume.

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

³ 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.

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Notes:

¹ The vacuum pump should be able to apply at least 400 mmHg pressure.

6. Insert the **25 ml Reservoir** into the **Spin-Away™ Filter** and place the assembly onto the vacuum manifold.¹
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 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 100 µl **DNase/RNase-Free Water** directly to the Spin-Away™ Filter. Incubate for 2 minutes and centrifuge. Discard the filter.
11. Add 200 µl **Cell-free Recovery Buffer** to the eluate and mix by pipetting.
12. Add 450 µl 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 µl **RNA Prep Buffer** to the column. Centrifuge and discard the flow-through.
15. Add 700 µl **RNA Wash Buffer** to the column. Centrifuge and discard the flow-through.
16. 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).
17. Add 15 µl **DNase/RNase-Free Water** directly to the column matrix, incubate for 2 minutes and centrifuge to elute total cell-free nucleic acid.²

The eluted total cell-free nucleic acid can be used immediately or stored at -70 °C.³

² Alternatively, for highly concentrated cfDNA/cfRNA, use ≥ 6 µl elution volume.

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

Appendix B: 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	<u>5 µl</u>
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 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).

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.

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	<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 starting the protocol is important.</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. Do not add more than the indicated amount of proteinase K and do not increase the digestion temperature above 37 °C. Increased vacuum pressures (≥ 400 mmHg) can help the lysate flow through the filter faster.</p>
Low Yield	<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 RNA binding. Ensure sample is completely digested before proceeding to sample binding.</p>
cfDNA/cfRNA Separation Efficiency	<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 < 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>

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Ordering Information

Product Description	Catalog No.	Kit size
Quick-cfDNA/cfRNA™ Serum & Plasma Kit	R1072	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-cfDNA/cfRNA™ Digestion Buffer	R1072-1-150	150 ml
Quick-cfDNA/cfRNA™ Binding Buffer	R1072-2-150	150 ml
Cell-free Recovery Buffer	R1072-3-20	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

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