INSTRUCTION MANUAL

Quick-cfDNA™ Serum & Plasma Kit

Catalog No. D4076

Highlights

- High-quality DNA, including cell-free, is easily and robustly purified from up to 10 ml of serum/plasma, up to 1 ml amniotic fluid or cerebrospinal fluid, or up to 1 ml saliva.
- Zymo-Spin™ technology enables elution of DNA in as little as 35 µl and ensures it is ready for all sensitive downstream applications such as qPCR and Next-Generation sequencing.

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Notes:

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

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.

[1, 2] Double centrifuged plasma is preferred for efficient depletion of residual cells, cell debris, and particulate matter when compared to plasma prepared using the single centrifugation methodology. Recommended plasma preparation methods:

a) Tuck MK, Chan DW, Chia D, *et al. Journal of Proteome Research*. 2009, **8**(1):113-117.

b) Warton *et al. BMC Genomics* 2014, **15**:476.

c) Peter B. Gahan. *Circulating Nucleic Acids in Early Diagnosis, Prognosis and Treatment Monitoring: An Introduction. pg. 55.*

Note – ™Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. 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 Contents

***Quick*-cfDNA™ Serum & Plasma Kit** (Kit Size)	**D4076** (50 preps.)	**Storage Temperature**
Proteinase K	4 x 125 mg	-20ºC (after mixing)
Proteinase K Storage Buffer	2 x 14 ml	Room Temp.
S&P 5X Digestion Buffer	2 x 32 ml	Room Temp.
S&P DNA Binding Buffer	4 x 170 ml	Room Temp.
S&P DNA Prep Buffer	2 x 10 ml	Room Temp.
S&P DNA Wash Buffer (concentrate)	2 x 12 ml	Room Temp.
DNA Elution Buffer	2 x 4 ml	Room Temp.
Zymo-Spin™ III-S Column Assembly	50	Room Temp.
Collection Tubes	50	Room Temp.
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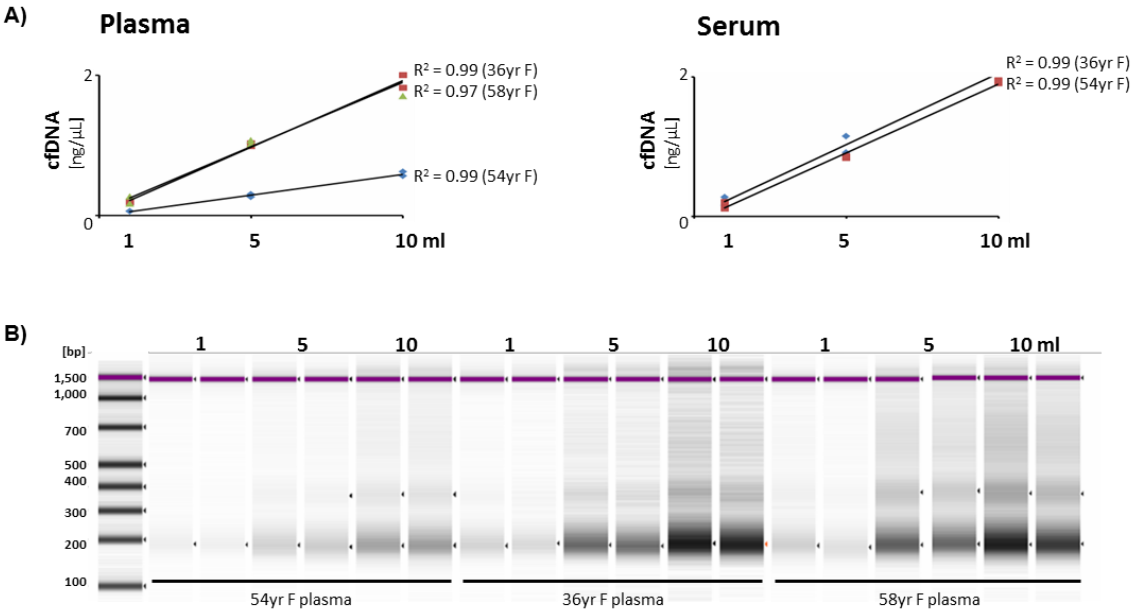
Specifications

- **Sample Sources** – Serum, plasma, amniotic fluid, and cerebrospinal fluid (CSF).
- **Processing volume**

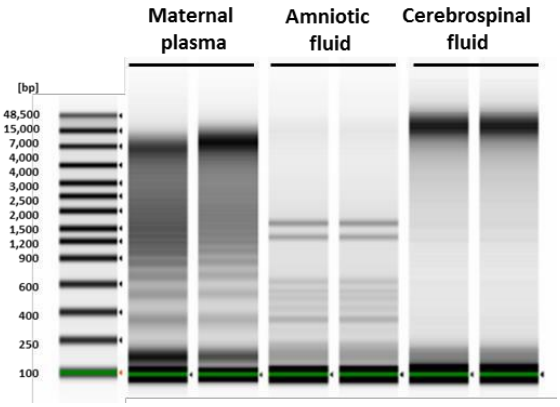
  Plasma – Single centrifugation[1]: up to 3 ml
  Double centrifugation[2]: up to 10 ml
  Serum – up to 10 ml
  Amniotic fluid – up to 1 ml
  Cerebrospinal fluid (CSF) – up to 1 ml
  Saliva – up to 1 ml
  Cell-free saliva – up to 5 ml
- **DNA Purity** – High-quality DNA is ready for all sensitive downstream applications such as qPCR and Next-Generation sequencing.
- **DNA Recovery** – Recover DNA ≥ 100bp (optimized for recovery of cell-free DNA). Yields can vary considerably among different individuals. Typically DNA recovery ranges from 1-100 ng/ml of plasma or serum. The yield varies depending on the sample source and the health of the donor.
- **Recovery Volume** – ≥ 35 µl of **DNA Elution Buffer** or DNase free water.
- **DNA Storage** – Eluted DNA should be stored at ≤ -20ºC.
- **Required Equipment** – Water bath or heat block (55ºC), microcentrifuge, vacuum/vacuum manifold or swinging bucket centrifuge.

Product description

***Quick*-cfDNA™ Serum & Plasma Kit** provides a simple and reliable method for the rapid preparation of high-quality circulating cell-free DNA from serum, plasma, amniotic fluid and cerebrospinal fluid (CSF)[1, 2]. A combination of chemical and enzymatic methods are used to efficiently recover total DNA (including cell-free apoptotic, necrotic, mitochondrial and viral DNA) linearly from up to 10 ml of sample (*Reference Figure A*). Zymo-Spin™ technology allows for ultra-pure DNA to be eluted in as little as 35 µl water[3]. The resulting DNA is suitable for all subsequent analyses and molecular manipulations such as qPCR, Next-Generation sequencing and DNA methylation analyses[4]. Zymo's serum and plasma cell-free DNA extraction technology will empower your discovery of circulating DNA biomarkers.



Cell-free DNA recovery scales proportionally with sample input using the *Quick*-cfDNA™ Serum & Plasma Kit. (A) Graphs and (B) gel image show the linear recovery of cfDNA from human plasma and serum (healthy female donors), as measured by Tapestation 2200 (Agilent, in duplicates).



Total DNA is efficiently purified from cell-free biological fluids with the *Quick*-cfDNA™ Serum & Plasma Kit. Total DNA, including both high and low molecular weight species, purified (duplicates) from human maternal plasma, amniotic fluid and cerebrospinal fluid was analyzed by Tapestation 2200 (Agilent).

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

Notes:

[1] For Urine samples, please use the *Quick*-DNA™ Urine kit (Catalog #: D3061).

[2] For viral DNA isolation from serum or plasma, use the ZR Serum DNA Kit™ (Catalog #: D3013) or *Quick*-DNA™ Universal Kit (Catalog #: D4068; D4069).

[3] To concentrate your DNA into as little as 6 µl, use the DNA Clean & Concentrator™-5 kit (Catalog #: D4003; D4004; D4013; D4014).

[4] Zymo Research offers the following for rapid and precise DNA methylation detection:

EZ DNA Methylation-Lightning™ Kit (D5030, D5031, D5032, D5033, D5046, D5047)

Notes:

Reagent Preparation

- ✓ Prior to use, add 6.5 ml of **Proteinase K Storage Buffer** to each **Proteinase K** (125 mg) tube. The final concentration of **Proteinase K** is ~20 mg/ml. Store at -20ºC after mixing.
- ✓ Prior to use, add 48 ml 95-100% ethanol to the 12 ml **S&P DNA Wash Buffer**.

Sample Processing Guide

Sample	Processing Volume	Protocol
Plasma, Serum	≤ 5 ml	Page 3
Plasma, Serum	5 - 10 ml	Page 6
Amniotic fluid, Cerebrospinal fluid	≤ 1 ml	Page 3
Saliva	≤ 1 ml	Page 7
Cell-free saliva	≤ 5 ml	Page 8

Protocol

Purification of cell-free DNA from ≤ 5 ml sample[1]

Unless otherwise indicated, perform all procedures at room temperature (15-30°C).

For sample input other than 200 µl, adjust **S&P 5X Digestion Buffer**, **Proteinase K** and **S&P DNA Binding Buffer** proportionally.

1. Add 50 µl of **S&P 5X Digestion Buffer** to every 200 µl of serum, plasma or biological fluid and mix thoroughly *(Reference Table 1)*.
2. Add 20 µl of **Proteinase K** to every 200 µl of serum, plasma or biological fluid and mix thoroughly *(Reference Table 1)*.
3. Incubate at 55 °C for 30 minutes.
4. Add two volumes of **S&P DNA Binding Buffer** to the digested sample from step 3 and mix thoroughly *(Reference Table 1)*.

Table 1: Quick Setup Guide

Sample Volume	200 µl	1 ml	3 ml	5 ml
S&P 5X Digestion Buffer	50 µl	250 µl	750 µl	1.25 ml
Proteinase K	20 µl	100 µl	300 µl	500 µl
Mix thoroughly and incubate at 55 ºC for 30 minutes.				
Add **S&P DNA Binding Buffer**	540 µl	2.7 ml	8.1 ml	13.5 ml

To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 5 for the centrifugation method.

[1] If you routinely process < 5 ml sample, in order to make full use of the reagents provided, you can purchase Zymo-Spin™ III-S columns separately (Catalog No. C1049-25).

Vacuum protocol

This product is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold[1].

5. Ensure the connection between the **Reservoir** and **Zymo-Spin™ III-S Column** are finger-tight and place the assembly onto a vacuum manifold.
6. Transfer the entire mixture (containing **S&P DNA Binding Buffer**) into **Zymo-Spin™ III-S Column Assembly**. Switch on the vacuum pump until the entire mixture has been completely drawn through the column. Switch off the vacuum pump and discard the top **Reservoir**.

   Alternatively, Steps 7-8 can be performed using a microcentrifuge.
7. With the vacuum off, add 400 µl **S&P DNA Prep Buffer** to the column. Switch on the vacuum pump until the liquid has been completely drawn through the column.
8. With the vacuum off, add 700 µl **S&P DNA Wash Buffer** to the column. Switch on the vacuum pump until the liquid has been completely drawn through the column. Repeat this wash step.
9. Transfer the column into a **Collection Tube** and centrifuge at full speed for 1 minute in a microcentrifuge to ensure complete removal of the wash buffer.
10. Transfer the **Zymo-Spin™ III-S Column** into a 1.5 ml DNase/RNase-free tube (not provided). Add ≥ 50 µl **DNA Elution Buffer** [2, 3] directly to the column matrix. Incubate at room temperature for 3 minutes and then centrifuge at maximum speed for 30 seconds in a microcentrifuge.

Eluted DNA can be used immediately for downstream applications or stored at ≤ -20°C.[4]

Note: *Generally, serum and plasma contain very low quantities of DNA and therefore Nanodrop is not recommended to quantify the DNA. High sensitivity techniques such as qPCR, Tapestation, Bioanalyzer, or Qubit, should be used to quantify and analyze the DNA.*

Notes:

[1] To achieve optimal performance, the vacuum pump should be able to apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove any residual lysate remaining in the matrix.

[2] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[3] The total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. If you load the column with 35 µL, it's recommended to load the eluate back to membrane, incubate for 3 minutes at room temperature, and centrifuge again.

[4] To concentrate your DNA into as little as 6 µl, use the DNA Clean & Concentrator™-5 kit (Catalog #: D4003; D4004; D4013; D4014).

Centrifugation Protocol

Perform steps 1-4 as indicated in the general protocol, see page 4.

5. Ensure the connection between the **Reservoir** and **Zymo-Spin™ III-S Column** is finger-tight and place the assembly into a 50 ml conical tube (not provided).
6. Transfer up to 10 ml of the mixture (containing **S&P DNA Binding Buffer**) into the **Zymo-Spin™ III-S Column Assembly** and centrifuge at 1,000 *x g* for 2 minutes. Discard the flow-through and repeat until the entire mixture has passed through the column.
7. Unscrew the orange Luer Lock cap from the top of the **Zymo-Spin™ III-S** column and discard the top **Reservoir**.
8. Place the Zymo-Spin™ III-S column in a **Collection Tube**. Add 400 µl **S&P DNA Prep Buffer** to the Zymo-Spin™ III-S column and centrifuge at ≥ 10,000 *x g* for 30 seconds in a microcentrifuge. Discard the flow-through.
9. Add 700 µl **S&P DNA Wash Buffer** to the Zymo-Spin™ III-S column and centrifuge at ≥ 10,000 *x g* for 30 seconds. Discard the flow-through.
10. Add 400 µl **S&P DNA Wash Buffer** to the Zymo-Spin™ III-S column and centrifuge at full speed for 1 minute to ensure complete removal of the wash buffer.
11. Transfer the column into a 1.5 ml DNase-free tube (not provided). Add ≥ 50 µl **DNA Elution Buffer** [1, 2] directly to the column matrix. Incubate at room temperature for 3 minutes and then centrifuge at maximum speed for 30 seconds.

Eluted DNA can be used immediately for downstream applications or stored at ≤ -20°C.[3]

Note: *Generally, serum and plasma contain very low quantities of DNA and therefore Nanodrop is not recommended to quantify the DNA. High sensitivity techniques such as qPCR, Tapestation, Bioanalyzer, or Qubit, should be used to quantify and analyze the DNA.*

Notes:

[1] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[2] The total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**. If you load the column with 35 µL, it's recommended to load the eluate back to membrane, incubate for 3 minutes at room temperature, and centrifuge again.

[3] To concentrate your DNA into as little as 6 µl, use the DNA Clean & Concentrator™-5 kit (Catalog #: D4003; D4004; D4013; D4014).

Purification of cell-free DNA from 5 – 10 ml sample

Before you start:

- ✓ *If you routinely process > 5 ml sample, in order to make full use of the columns provided, you can purchase an additional buffer set (Catalog No. D4076-A) or individual reagents (Please refer to page 9 for ordering information).*

Protocol

1. Add 250 µl of **S&P 5X Digestion Buffer** per ml of serum, plasma or biological fluid and mix thoroughly *(Reference Table 2)*.
2. Add 100 µl of **Proteinase K** per ml of serum, plasma or biological fluid and mix thoroughly *(Reference Table 2)*.
3. Incubate at 55 °C for 30 minutes.
4. Add two volumes of **S&P DNA Binding Buffer** to the digested sample from step 3 and mix thoroughly *(Reference Table 2)*.

Table 2: Quick Setup Guide

Sample Volume	1 ml	6 ml	8 ml	10 ml
S&P 5X Digestion Buffer	250 µl	1.5 ml	2 ml	2.5 ml
Proteinase K	100 µl	600 µl	800 µl	1 ml
Mix thoroughly and incubate at 55 ºC for 30 minutes.				
Add **S&P DNA Binding Buffer**	2.7 ml	16.2 ml	21.6 ml	27 ml

5. Ensure the connection between the **Reservoir** and **Zymo-Spin™ III-S Column** is finger-tight and place the assembly into a 50 ml conical tube.
6. Transfer 10 ml of the mixture from step 4 into the **Zymo-Spin™ III-S Column Assembly** and centrifuge at 1,000 *x g* for 2 minutes[1]. Discard the flow-through and repeat until the entire mixture has passed through the column.
7. Unscrew the orange Luer Lock cap from the top of the **Zymo-Spin™ III-S** column and discard the top **Reservoir**.
8. Place the Zymo-Spin™ III-S column in a **Collection Tube**. Add 400 µl **S&P DNA Prep Buffer** to the Zymo-Spin™ III-S column and centrifuge at ≥ 10,000 *x g* for 30 seconds in a microcentrifuge. Discard the flow-through.
9. Add 700 µl **S&P DNA Wash Buffer** to the Zymo-Spin™ III-S column and centrifuge at ≥ 10,000 *x g* for 30 seconds. Discard the flow-through.
10. Add 400 µl **S&P DNA Wash Buffer** to the Zymo-Spin™ III-S column and centrifuge at full speed for 1 minute to ensure complete removal of the wash buffer.
11. Transfer the column into a 1.5 ml DNase-free tube (not provided). Add ≥ 50 µl **DNA Elution Buffer** [2, 3, 4] directly to the column matrix. Incubate at room temperature for 3 minutes and then centrifuge at maximum speed for 30 seconds.

Notes:

[1] Although centrifugation method is recommended, the vacuum manifold can be used.

[2] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If water is used, make sure the pH is > 6.0.

[3] The total yield may be improved by eluting the DNA with 60-70 ºC **DNA Elution Buffer**. If you load the column with 35 µL, it's recommended to load the eluate back to membrane, incubate for 3 minutes at room temperature, and centrifuge again.

[4] To concentrate your DNA into as little as 6 µl, use the DNA Clean & Concentrator™-5 kit (Catalog #: D4003; D4004; D4013; D4014).

Notes:

Appendix A

Purification of total (cellular and cell-free) DNA from Saliva

If only cell-free DNA is desired, please refer to page 8.

Protocol

1. Transfer up to 1 ml saliva into a microcentrifuge tube or a conical tube.
   Note: *Use a microcentrifuge tube if processing ≤ 200 µl saliva.*
   Use a conical tube if processing > 200 µl saliva.
2. Add 0.5 ml of **S&P 5X Digestion Buffer** per ml saliva and mix thoroughly *(Reference Table 3).*
3. Add 0.5 ml of dd H_2O per ml saliva and mix thoroughly *(Reference Table 3)*.
4. Add 100 µl of **Proteinase K** per ml saliva and mix thoroughly *(Reference Table 3).*
5. Incubate at 55 °C for 30 minutes.
6. Add one volume of **S&P DNA Binding Buffer** to the digested sample from step 3 and mix thoroughly *(Reference Table 3)*.
7. Add one volume of 95%-100% ethanol to the new mixture from step 5 and mix thoroughly *(Reference Table 3)*.

Table 3: Quick Setup Guide

Saliva Volume	200 µl	500 µl	750 µl	1 ml
S&P 5X Digestion Buffer	100 µl	250 µl	375 µl	0.5 ml
dd H_2O	100 µl	250 µl	375 µl	0.5 ml
Proteinase K	20 µl	50 µl	75 µl	100 µl
Mix thoroughly and incubate at 55 ºC for 30 minutes.				
Add **S&P DNA Binding Buffer**	420 µl	1.05 ml	1.58 ml	2.1 ml
Add ethanol	840 µl	2.1 ml	3.15 ml	4.2 ml

To continue processing the lysate using the recommended vacuum protocol, proceed to the page 4. If a vacuum is not available, proceed to page 5 for the centrifugation method.

Appendix B

Purification of cell-free DNA from saliva

Protocol

1. Dilute the starting saliva sample with equal volume of isotonic solution (e.g. 1X PBS), centrifuge the diluted saliva sample in a microcentrifuge tube or a conical tube at 5,000 *x g* for 10 minutes to remove cells.
2. Without disturbing the loose cell pellet, carefully transfer saliva supernatant to a new microcentrifuge tube or a conical tube.

   Note: The cellular pellet can be processed separately[1] or discarded.
3. Transfer up to 5 ml saliva supernatant into a microcentrifuge tube or a conical tube.

   Note: Use a microcentrifuge tube if processing ≤ 200 µl cell-free saliva supernatant.

   Use a conical tube if processing > 200 µl cell-free saliva supernatant.
4. Add 0.5 ml of **S&P 5X Digestion Buffer** per ml saliva supernatant and mix thoroughly *(Reference Table 4)*.
5. Add 0.5 ml of dd H_2O per ml saliva supernatant and mix thoroughly *(Reference Table 4).*
6. Add 100 µl of **Proteinase K** per ml saliva supernatant and mix thoroughly *(Reference Table 4)*.
7. Incubate at 55 °C for 30 minutes.
8. Add <u>one volume</u> of **S&P DNA Binding Buffer** to the digested sample from step 5 and mix thoroughly *(Reference Table 4)*.
9. Add <u>one volume</u> of 95%-100% ethanol to the new mixture from step 7 and mix thoroughly *(Reference Table 4)*.

Table 4: Quick Setup Guide

Saliva supernatant Volume	200 µl	1 ml	3 ml	5 ml
S&P 5X Digestion Buffer	100 µl	0.5 ml	1.5 ml	2.5 ml
dd H_2O	100 µl	0.5 ml	1.5 ml	2.5 ml
Proteinase K	20 µl	100 µl	300 µl	500 µl
Mix thoroughly and incubate at 55 ºC for 30 minutes.				
Add **S&P DNA Binding Buffer**	420 µl	2.1 ml	6.3 ml	10.5 ml
Add ethanol	840 µl	4.2 ml	12.6 ml	21.0 ml

To continue processing the lysate using the recommended vacuum protocol, proceed to the page 4. If a vacuum is not available, proceed to page 5 for the centrifugation method.

Notes:

[1] To process the cellular pellet:

a) Resuspend the pellet in 200 µl isotonic solution.
b) Add 100 µl of **S&P 5X Digestion Buffer**.
c) Add 100 µl of dd H_2O.
d) Add 20 µl of **Proteinase K** and mix thoroughly.
e) Proceed to step 7.

Appendix C

Isolation of short (< 100 bp) cell-free DNA

PROTOCOL

1. Transfer up to 5 ml plasma into a microcentrifuge tube or a conical tube.
2. Add 250 µl of **S&P 5X Digestion Buffer** per ml plasma and mix thoroughly *(Reference Table 5).*
3. Add 100 µl **Proteinase K** per ml plasma and mix thoroughly. *(Reference Table 5)*.
4. Incubate at 55 °C for 30 minutes.
5. Add two volumes of **S&P DNA Binding Buffer** to the digested sample from step 3 and mix thoroughly *(Reference Table 5)*.
6. Add 0.7 ml of 95%-100% ethanol per ml plasma to the new mixture from step 5 and mix thoroughly *(Reference Table 5)*.

Table 5: Quick Setup Guide

Sample Volume	200 µl	1 ml	3 ml	5 ml
S&P 5X Digestion Buffer	50 µl	250 µl	750 µl	1.25 ml
Proteinase K	20 µl	100 µl	300 µl	500 µl
Mix thoroughly and incubate at 55 ºC for 30 minutes.				
Add **S&P DNA Binding Buffer**	540 µl	2.7 ml	8.1 ml	13.5 ml
Add ethanol	280 µl	0.7 ml	2.1 ml	3.5 ml

To continue processing the lysate using the recommended vacuum protocol, proceed to the page 4. If a vacuum is not available, proceed to page 5 for the centrifugation method.

Ordering Information[1]

Product Description	Catalog No.	Kit Size
***Quick*-cfDNA™ Serum & Plasma kit**	D4076	50 Preps.

For Individual Sale	Catalog No.	Amount
Quick*-cfDNA™ Serum & Plasma Buffer Set If sample inputs of > 5 ml are routinely processed, the *Quick*-cfDNA™ Serum & Plasma Buffer Set is available as a refill. ** Buffers only, columns not included.* This buffer set includes the following reagents: **Proteinase K** 4 x 125 mg **Proteinase K Storage Buffer** 2 x 14 ml **S&P 5X Digestion Buffer** 2 x 32 ml **S&P DNA Binding Buffer** 4 x 170 ml **S&P DNA Prep Buffer** 2 x 10 ml **S&P DNA Wash Buffer** (concentrate) 2 x 12 ml	D4076-A	50 preps.
S&P 5X Digestion Buffer	D4076-1-32	32 ml
S&P DNA Binding Buffer	D4076-2-170	170 ml
S&P DNA Prep Buffer	D4076-3-10	10 ml
S&P DNA Wash Buffer	D4076-4-12	12 ml
Zymo-Spin™ III-S Column Assembly	C1049-25	25 columns
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1000 tubes
DNA Elution Buffer	D3004-4-1 D3004-4-4 D3004-4-10 D3004-4-50	1 ml 4 ml 10 ml 50 ml

Notes:

[1] You can place your order by the following methods:

1) Online Orders: www.zymoresearch.com
2) Email Orders: orders@zymoresearch.com
3) Fax Orders: (949)266-9452
4) Phone Orders: (888)882-9682 (Toll Free USA Only)