omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Circulating DNA Kit

D3091-00	5 preps
D3091-01	50 preps

Manual Date: June 2021
Revision Number: v6.2

For Research Use Only

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Table of Contents

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Introduction and Overview

E.Z.N.A.® Circulating DNA Kit provides a rapid and easy method for the isolation of Circulating DNA from plasma, serum, and other acellular body fluids. Samples can be either fresh or frozen, provided that they have not undergone more than one freeze/thaw cycle. DNA purified using the E.Z.N.A® Circulating DNA method is ready for applications such as PCR, Next Generation Sequencing, and genotyping.

E.Z.N.A.® Circulating DNA Kit uses the reversible nucleic acid binding properties of our HiBind® matrix combined with the speed of mini column centrifugation. A specially formulated buffer system allows circulating DNA to bind to the HiBind® matrix. Samples are lysed under denaturing conditions and then transferred to the E.Z.N.A. Circulating Column where DNA binds and cellular debris, hemoglobin, and other proteins are washed away.

New in this Edition:

August 2019
- SPW Wash Buffer has been renamed SPW Buffer. This is a name change only. The formulation has not changed.

July 2017
- Buffer volumes have been updated to more accurately reflect usage.

April 2017
- The buffer system has been updated to improve overall kit performance. DCL Buffer, ACX Buffer, and DNA Wash Buffer are no longer provided in this kit. DS Buffer, JSB Buffer, and SPW Wash Buffer have been added to the kit.

Kit Contents

Product	D3091-00	D3091-01
Circulating DNA Mini Columns	5	50
#6 Column Funnel	5	50
2 mL Collection Tubes	5	50
DS Buffer	1.5 mL	20 mL
JSB Buffer	25 mL	225 mL
VHB Buffer	4.4 mL	44 mL
SPW Buffer	2.5 mL	25 mL
Elution Buffer	15 mL	125 mL
Proteinase K Solution	350 µL	4 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® Circulating DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. For long-term storage, store Proteinase K Solution at 2-8°C. During shipment or storage in cool ambient conditions, precipitates may form in VHB Buffer, JSB Buffer, and/or DS Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

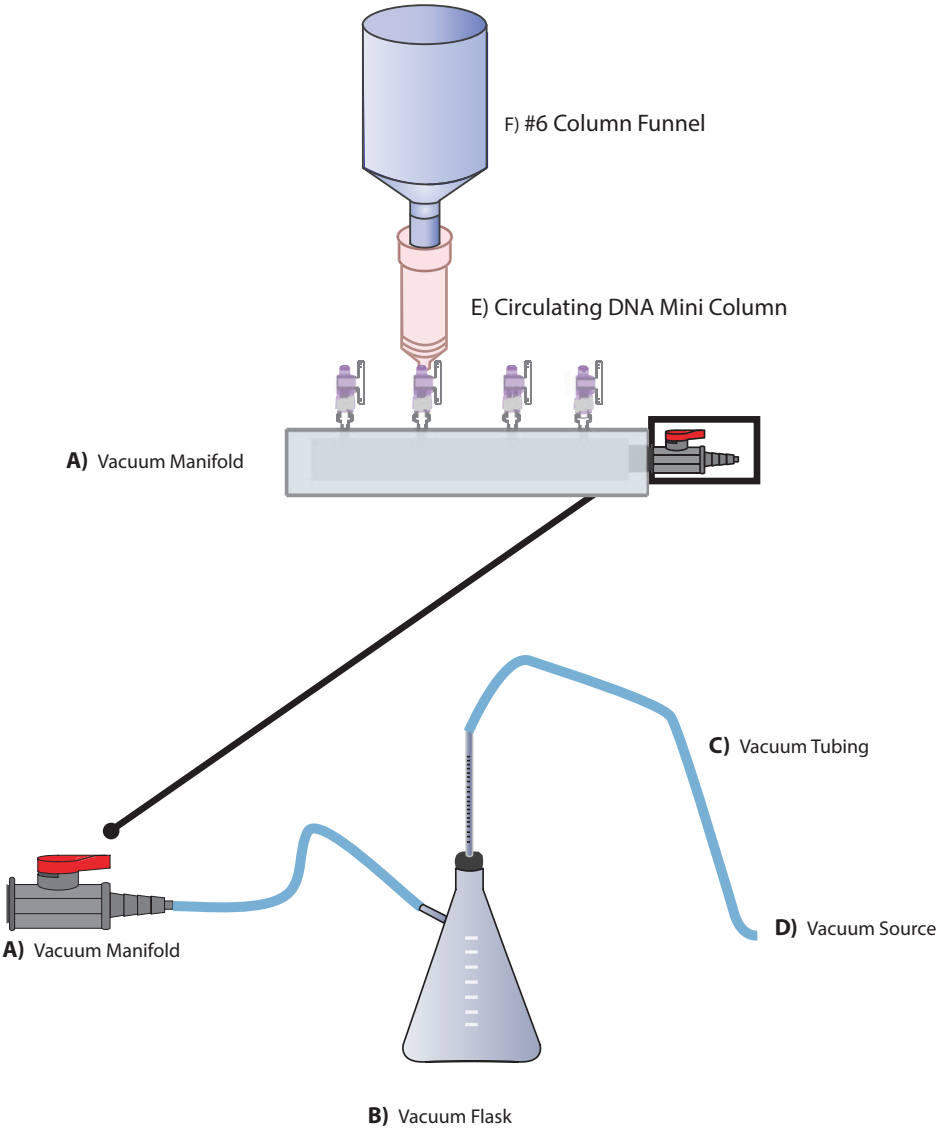
- Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3091-00	5.6 mL
D3091-01	56 mL

- Dilute SPW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3091-00	10 mL
D3091-01	100 mL

Vacuum Manifold Set Up



E.Z.N.A.® Circulating DNA Kit - Protocol for 1000-2000 µL Samples

Materials and Equipment to be Supplied by User:

- Vacuum manifold
- Microcentrifuge capable of ≥13,000 x *g*
- Water bath, incubator, or oven capable of 60°C
- Vortexer
- Nuclease-free 15 mL centrifuge tubes
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 100% ethanol

Before Starting:

- Prepare VHB Buffer and SPW Buffer according to the "Preparing Reagents" section on Page 4
- Set an incubator to 60°C
- Heat Elution Buffer to 60°C

Note: The following protocol is designed for 1000-2000 µL plasma or serum samples. The buffer volumes in Steps 2-5, can be scaled up or down accordingly. Keep the VHB Buffer and SPW Buffer the same.

1. Add 1000-2000 µL plasma or serum to a 15 mL centrifuge tube (not provided). Choose the correct plasticware depending on the magnetic stand being utilized to process the samples. Bring the sample volume up to 2 mL with Elution Buffer (provided) if less than 2 mL.

2. Add 30 µL Proteinase K Solution.

3. Add 135 µL DS Buffer. Vortex at maximum speed or pipet up and down to mix thoroughly.

4. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

5. Add 2 mL JSB Buffer. Vortex at maximum speed for 30 seconds to mix thoroughly.

6. Insert a #6 Column Funnel into a Circulating DNA Mini Column. Then connect the Circulating DNA Mini Column/#6 Column Funnel assembly to a vacuum manifold. See Page 5 for an illustration.

7. Transfer the sample from Step 5 to the Circulating DNA Mini Column/#6 Column Funnel assembly.

8. Switch on the vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Remove the #6 Column Funnel from the Circulating DNA Mini Column leaving the Circulating DNA Mini Column inserted into the vacuum manifold.

11. Add 700 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 4.

12. Switch on the vacuum source to draw the VHB Buffer through the column.

13. Turn off the vacuum.

14. Repeat Steps 11-13 for a second VHB Buffer wash step.

15. Add 700 µL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 4.

16. Switch on the vacuum source to draw the SPW Buffer through the column.

17. Turn off the vacuum.

18. Repeat Steps 15-17 for a second SPW Buffer wash step.

19. Insert the Circulating DNA Mini Column into a 2 mL Collection Tube (provided).

20. Centrifuge at maximum speed for 5 minutes to completely dry the Circulating DNA Mini Column.

    Note: It is important to dry the Circulating DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the Circulating DNA Mini Column to a nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not provided).

22. Add 30-150 µL Elution Buffer heated to 60°C.

23. Let sit for 5 minutes at room temperature.

24. Centrifuge at 13,000 x *g* for 1 minute.

25. Store the eluted DNA at -20°C.

E.Z.N.A.® Circulating DNA Kit - Protocol for 2000-4000 µL Samples

Materials and Equipment to be Supplied by User:

- Vacuum manifold
- Microcentrifuge capable of ≥13,000 x *g*
- Water bath, incubator, or oven capable of 60°C
- Vortexer
- Nuclease-free 50 mL centrifuge tubes
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 100% ethanol

Before Starting:

- Prepare VHB Buffer and SPW Buffer according to the "Preparing Reagents" section on Page 4
- Set an incubator to 60°C
- Heat Elution Buffer to 60°C

Note: The following protocol is designed for 2000-4000 µL plasma or serum samples. The buffer volumes in Steps 2-5 can be scaled up or down accordingly. Keep the VHB Buffer and SPW Buffer volumes the same.

1. Add 2000-4000 µL plasma or serum to a 50 mL centrifuge tube (not provided). Bring up the volume to 4 mL using Elution Buffer (provided) if the sample volume is less than 4 mL.

2. Add 60 µL Proteinase K Solution.

3. Add 270 µL DS Buffer. Vortex at maximum speed or pipet up and down to mix thoroughly.

4. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

5. Add 4 mL JSB Buffer. Vortex at maximum speed or pipet up and down to mix thoroughly.

6. Insert a #6 Column Funnel into a Circulating DNA Mini Column. Then connect the Circulating DNA Mini Column/#6 Column Funnel assembly to a vacuum manifold. See Page 5 for an illustration.

7. Transfer the sample from Step 5 to the Circulating DNA Mini Column/#6 Column Funnel assembly.

8. Switch on the vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Remove the #6 Column Funnel from the Circulating DNA Mini Column leaving the Circulating DNA Mini Column inserted into the vacuum manifold.

11. Add 700 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 4.

12. Switch on the vacuum source to draw the VHB Buffer through the column.

13. Turn off the vacuum.

14. Repeat Steps 11-13 for a second VHB Buffer wash step.

15. Add 700 µL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see the instructions in the "Preparing Reagents" section on Page 4.

16. Switch on the vacuum source to draw the SPW Buffer through the column.

17. Turn off the vacuum.

18. Repeat Steps 15-17 for a second SPW Buffer wash step.

19. Insert the Circulating DNA Mini Column into a 2 mL Collection Tube (provided).

20. Centrifuge at maximum speed for 5 minutes to completely dry the Circulating DNA Mini Column.

    Note: It is important to dry the Circulating DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the Circulating DNA Mini Column to a nuclease-free 1.5 mL or 2 mL microcentrifuge tube (not provided).

22. Add 30-150 µL Elution Buffer heated to 60°C.

23. Let sit for 5 minutes at room temperature.

24. Centrifuge at 13,000 x *g* for 1 minute.

25. Store the eluted DNA at -20°C.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Proteinase K Solution, 10 mL	AC116
VHB Buffer, 440 mL	VHB-440
SPW Buffer, 25 mL	PDR045
Elution Buffer, 100 mL	PDR048
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00

Notices & Disclaimers

For European Union Use.

JSB Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or in vitro diagnostics.

For more purification solutions, visit **www.omegabiotek.com**

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