E.Z.N.A.® Circulating DNA Kit

D3091-00	5 preps
D3091-01	50 preps
D3091-02	200 preps

July 2016

E.Z.N.A.® Circulating DNA Kit

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OMEGA bio-tek

Innovations in nucleic acid isolation

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.

Kit Contents

Product	D3091-00	D3091-01	D3091-02
Circulating DNA Mini Columns	5	50	200
#6 Column Funnel	5	50	200
2 mL Collection Tubes	5	50	200
DCL Buffer	20 mL	200 mL	4x200 mL
ACX Buffer	16 mL	2x80 mL	8x80 mL
VHB Buffer	2.2 mL	22 mL	88 mL
DNA Wash Buffer	2 mL	20 mL	2x40 mL
Elution Buffer	12 mL	120 mL	2x240 mL
Proteinase K Solution	2.2 mL	22 mL	88 mL
Carrier RNA Solution	100 µL	600 µL	2.4 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Circulating DNA Kit components are guaranteed for at least 24 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 and/or ACX Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

- Dilute ACX Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D3091-00	24 mL
D3091-01	120 mL per bottle
D3091-02	120 mL per bottle

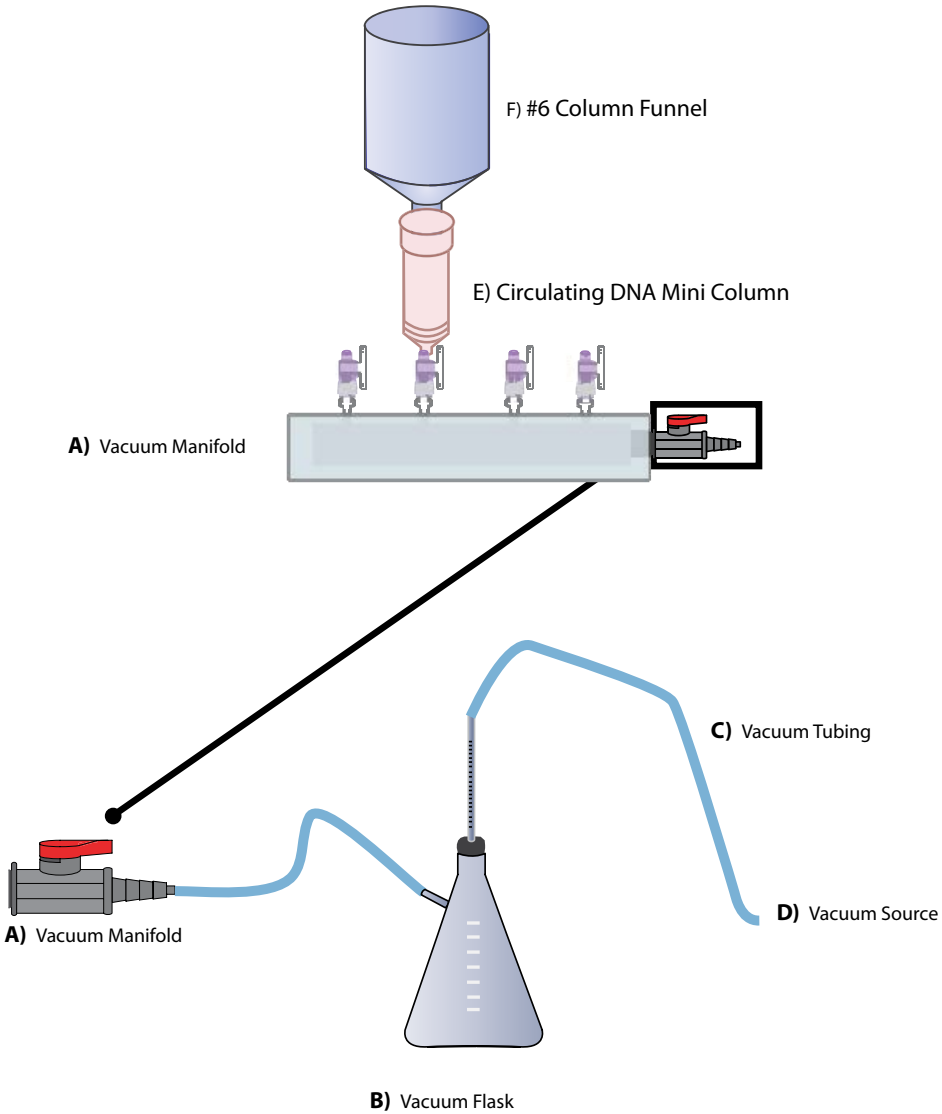
- Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3091-00	2.8 mL
D3091-01	28 mL
D3091-02	112 mL

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

Kit	100% Ethanol to be Added
D3091-00	8 mL
D3091-01	80 mL
D3091-02	160 mL per bottle

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
- Ice bucket
- Nuclease-free 15 mL centrifuge tubes
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 100% ethanol
- 100% isopropanol

Before Starting:

- Prepare ACX Buffer, VHB Buffer, and DNA Wash Buffer according to the "Preparing Reagents" section on Page 4
- Prepare an ice bucket
- 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, except for Carrier RNA Solution, can be scaled up or down accordingly. Keep the Carrier RNA Solution, VHB Buffer, and DNA Wash Buffer the same.

1. Add 1000-2000 µL plasma or serum to a 15 mL centrifuge tube (not provided).

2. Bring up the volume to 2000 µL using Elution Buffer.

   Note: If the sample volume was 1500 µL, add 500 µL Elution Buffer.

3. Add 200 µL Proteinase K Solution.

4. Add 1.6 mL DCL Buffer and 10 µL Carrier RNA Solution. Vortex at maximum speed for 30 seconds to mix thoroughly.

5. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 3.6 mL ACX Buffer. Vortex at maximum speed for 30 seconds to mix thoroughly.

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

7. Let sit on ice for 5 minutes.

8. 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.

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

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

11. Turn off the vacuum.

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

13. 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.

14. Switch on the vacuum source to draw the liquid through the column.

15. Turn off the vacuum.

16. Add 700 µL DNA Wash Buffer.

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

17. Switch on the vacuum source to draw the liquid through the column.

18. Turn off the vacuum.

19. Repeat Steps 16-18 for a second wash with DNA Wash Buffer.

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

21. 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.

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

23. Add 50-150 µL Elution Buffer heated to 60°C.

24. Let sit for 5 minutes at room temperature.

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

26. 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
- Ice bucket
- Nuclease-free 50 mL centrifuge tubes
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 100% ethanol
- 100% isopropanol

Before Starting:

- Prepare ACX Buffer, VHB Buffer, and DNA Wash Buffer according to the "Preparing Reagents" section on Page 4
- Prepare an ice bucket
- 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, except for Carrier RNA Solution, can be scaled up or down accordingly. Keep the Carrier RNA Solution, VHB Buffer, and DNA Wash Buffer volumes the same.

1. Add 2000-4000 µL plasma or serum to a 50 mL centrifuge tube (not provided).

2. Bring up the volume to 4000 µL using Elution Buffer.

   Note: If the sample volume was 2500 µL, add 1500 µL Elution Buffer.

3. Add 400 µL Proteinase K Solution.

4. Add 3.2 mL DCL Buffer and 10 µL Carrier RNA Solution. Vortex at maximum speed for 30 seconds to mix thoroughly.

5. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.

6. Add 7.2 mL ACX Buffer. Vortex at maximum speed for 30 seconds to mix thoroughly.

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

7. Let sit on ice for 5 minutes.

8. 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.

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

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

11. Turn off the vacuum.

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

13. 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.

14. Switch on the vacuum source to draw the liquid through the column.

15. Turn off the vacuum.

16. Add 700 µL DNA Wash Buffer.

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

17. Switch on the vacuum source to draw the liquid through the column.

18. Turn off the vacuum.

19. Repeat Steps 16-18 for a second wash with DNA Wash Buffer.

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

21. 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.

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

23. Add 50-150 µL Elution Buffer heated to 60°C.

24. Let sit for 5 minutes at room temperature.

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

26. 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
DNA Wash Buffer, 100 mL	PS010
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