Problem	Possible Cause	Suggestions
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation – it will not interfere with PCR or restriction digests.
	Hemoglobin remains on column	After application of sample to column, wash once with 300 µl Buffer AL.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer ACL	Mix thoroughly with Buffer BL prior to loading HiBind™ column.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Washing leaves colored residue in column	Incomplete lysis due to improper mixing with Buffer ACL	Buffer BL is viscous and the sample must be mixed thoroughly.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.

Contents

B. Vacuum Protocol: Purification of Circulating DNA from Plasma or Serum

Material and equipments supplied by user

- **Tabletop microcentrifuge and sterile 1.5 ml tubes**
- **Vacuum Manifold**
- **Water bath -** set to 65ºC
- **Ethanol -**approximately 0.3 ml per sample.
- **RNase A** - Prepare a stock solution of RNase A at 50mg/ml.

1. Prepare the lysate by following step 1-6 of Protocol A, Spin protocol on page 4.

2. Insert the HiBind® DNA Micro column into the vacuum manifold. Carefully apply the lysate to an HiBind® DNA column. Turn on the vacuum source to draw all liquid through the column. when all lysates have been drawn through the column completely, switch off the vacuum pump.

   Note: If the lysate has difficulty to pass through the column at this stage. Place the column into a collection tube (supplied). Close the lid and centrifuge at 8000 x g for 5 minutes or until all liquid pass through the column. Place the column into another collection tube (supplied) and continue step 7 of the spin protocol.

3. Pipet 700 µl of Buffer ACW1 into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.

4. Wash the column by pipetting 700 µl of DNA Wash Buffer diluted with ethanol into the column. Turn on the vacuum source to draw all liquid through the column. Turn off the vacuum.

5. Close the lid of HiBind® DNA column, remove it from the vacuum manifold. Insert the column into a collection tube (supplied) and centrifuge at 15,000 x g for 2 minute to completely dry the column.

6. Elute DNA as Step 13-15 on page 5.

Kit Contents

Product No.	D3091-00	D3091-01	D3091-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ DNA Micro columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer ACL	5 ml	50 ml	200 ml
Buffer ACB	10 ml	100 ml	2 x 200 ml
Buffer ACW1	5 ml	50 ml	150 ml
Carrier RNA	310 µg	310 µg	2 x 1 mg
DNA Wash Buffer	5 ml	20 ml	3 x 20 ml
Elution Buffer	5 ml	40 ml	160 ml
OB Protease	10 mg	100 mg	4 x 100mg
Protease Storage Buffer	1 ml	6 ml	24 ml
User Manual	1	1	1

Before Starting

IMPORTANT

1 Reconstitute OB Protease in 500 µl (5 preps), 5 ml (50preps) or 4 x 5 ml (200 preps) Protease Storage Buffer. Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20ºC.

2 DNA Wash Buffer Concentrate must be diluted with absolute ethanol (96-100%) as follows:

D3091-00 Add 20 ml absolute ethanol

D3091-01 Add 80 ml absolute ethanol

D3091-02 Add 80 ml absolute ethanol per bottle

Store diluted DNA Wash Buffer at room temperature

All centrifugation steps must be carried out at room temperature.

Introduction

E.Z.N.A.® Circulating DNA Kit provides a rapid and easy method for the isolation of Circulating DNA from plasma, serum, and other cell-free body fluids. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. The kit allows single or multiple, simultaneous processing of samples in under 120 minutes. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or ethanol, are eliminated. DNA purified using the E.Z.N.A® Circulating DNA method is ready for applications such as PCR, Circulating detection, and genotyping.

E.Z.N.A.® Circulating DNA Kit uses the reversible nucleic acid-binding properties of HiBind® matrix, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows Circulating DNA bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® DNA spin columns to which DNA binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality DNA is finally eluted in sterile deionized water or low salt buffer.

Storage and Stability

All components of the E.Z.N.A.® Circulating DNA Kit, except the OB Protease should be stored at 22ºC-25ºC. Once reconstituted in water, OB Protease must be stored at -20ºC. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. Under cool ambient conditions, a precipitate may form in the Buffer ACL and ACB. In case of such an event, heat the bottle at 37ºC to dissolve. Store Buffer ACL and ACB at room temperature.

Expiration Date: All E.Z.N.A.® Circulating DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25ºC.

Binding Capacity

Each HiBind ® column can bind approximately 20 µg DNA.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. Dilute the DNA in TE buffer and calculate concentration as:

[DNA] = (Absorbance$_{260}$) x (0.05 µg/µl*) x (Dilution factor)*

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Add the correct volume of Buffer BL and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 min.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 4). Incubation of column at 70°C for 5 min with Elution Buffer may increase yields.
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 5 before use.

A. Spin Protocol: Purification of Circulating DNA from 0.1-1ml Plasma or Serum

Materials and equipments Supplied by User

- **Tabletop microcentrifuge and sterile 1.5 ml tubes.**
- **Water bath -** set to 65ºC.
- **Ethanol -** approximately 0.3 ml per sample.
- **RNase A -** Prepare a stock solution of RNase A at 50 mg/ml.

NOTE: The procedure below has been optimized for use with FRESH or FROZEN Plasma or Serum samples from0.1 to 1 ml in volume. Other Cell-free samples can also be used. For DNA extraction from Blood, we suggest using the **E.Z.N.A.® Blood DNA Kit** (product number **D3392**). To isolate Circulating RNA from serum or other non-cellular body fluids use **E.Z.N.A.® Circulating RNA Kit**.

Preheat an aliquot of Elution Buffer (approximately 0.1 ml per sample) at 65ºC. **Carry out all centrifugation steps at room temperature**.

1. **Add ■25 µl OB Protease, ●50 µl OB Protease, or ✠ 100 µl OB Protease to** a sterile microcentrifuge tube.

2. **Add ■250 µl, ●500 µl, or ✠ 1ml plasma or Serum** to the tube containing protease.

3. **Add ■ 200 µl ACL Buffer and 5.6 µl Carrier RNA, ●400 µl ACL Buffer and 5.6 µl Carrier RNA, or ✠ 0.8ml Buffer ACL and 5.6 µl Carrier RNA.** Vortex at maxi speed for 30s to mix thoroughly.

4. Incubate at 60oC for 30 min.

5. **Add ■ 450 µl ACB Buffer, ●900 µl ACB Buffer, or ✠ 1.8ml Buffer ACB.** Vortex at maxi speed for 30s to mix thoroughly.

6. Incubate the mixture on ice for 10 min.

7. Assemble an HiBind® DNA Micro column in a 2 ml collection tube (provided).

8. **Transfer 750 µl of the lysate from step 6 into the column.** Centrifuge at 8,000 x g for 1 min to bind DNA. Discard flow-through liquid and assembe the column into the same collection tube.

9. Repeat step 8 until all of the lysate pass through the Micro column.

10. Place the column into a **second 2 ml tube** (provided) and wash by pipetting 700µl of Buffer ACW1. Centrifuge at 8,000 x g for 1 min. Again, Discard flow-through liquid and reuse the collection tube for next step.

11. Place the column into a **same 2 ml tube** from step 10 and wash by pipetting 700 µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

    Note that DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle or page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use.

12. Using a **new collection tube**, wash the column with a second 700 µl of DNA Wash Buffer and centrifuge as above. Discard flow-through and re-use the collection tube for next step.

13. Place the empty column into the same 2 ml collection tube form step 12, centrifuge at maximum speed (13,000 x g) for 2 min to dry the column. **This step is crucial for ensuring optimal elution in the following step**.

14. Place the column into a sterile 1.5 ml microfuge tube and add 20-50 µl of preheated (65ºC) Elution Buffer. Allow tubes to sit for 5 min at room temperature.

15. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Retain flow-through containing the DNA. Place column into a second 1.5 ml tube. Elute DNA again as step 11-12. Discard column and store the eluted DNA at -20°C.