

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 $\mu$ l Buffer DCL.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer DCL	Mix thoroughly with Buffer DCL prior to loading HiBind™ column.
	No ethanol added to Wash Buffer Concentrate.	Dilute DNA 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 DCL	Buffer DCL is viscous and the sample must be mixed thoroughly.
	No ethanol added to DNA Buffer Concentrate.	Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use.

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## 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

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 10 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. **Wash the column by pipetting another 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.
6. Close the lid of HiBind® DNA column, remove it from the vacuum manifold. Insert the column into a collection tube (supplied) and centrifuge at 13,000 x g for 5 minute to completely dry the column.
7. Elute DNA as Step 14-15 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 DCL	10 ml	50 ml	200 ml
Buffer ACB	20 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
Nuclease-Free Water	5 ml	40 ml	160 ml
OB Protease	600 µl	6 ml	24 ml
User Manual	1	1	1

## Before Starting

<b>IMPORTANT</b>	1	<b>Reconstitute Carrier RNA in Nuclease-Free Water at final concentration of 1mg/ml.</b> Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -80°C.
	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 <i>Store diluted DNA Wash Buffer at room temperature</i>

## 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 Carrier RNA should be stored at 22°C-25°C. Once reconstituted in water, Carrier RNA should be stored at -20°C. For long-term storage, OB Protease can be stored at 4°C. 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 DCL and ACB. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer DCL 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 22-25°C.

## 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:

$$[DNA] = (Absorbance_{260}) \times (0.05 \mu\text{g}/\mu\text{l}) \times (Dilution \text{ factor})$$

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 DCL 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 Nucleic-free water may increase yields.
	Improper washing	DNA 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 60°C.

**NOTE:** The procedure below has been optimized for use with FRESH or FROZEN Plasma or Serum samples from 0.1 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.<sup>®</sup> Blood DNA Kit (product number D3392). To isolate Circulating RNA from serum or other non-cellular body fluids use E.Z.N.A.<sup>®</sup> Circulating RNA Kit.

Preheat an aliquot of Nuclease-Free Water (approximately 0.1 ml per sample) at 60°C. 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 ■ 250µl, ● 500µl, or ☒ 1ml plasma or Serum to the tube containing OB Protease.
3. Add ■ 200µl DCL Buffer and 5.6 µl Carrier RNA, ● 400 µl DCL Buffer and 5.6 µl Carrier RNA, or ☒ 0.8ml Buffer DCL and 5.6 µl Carrier RNA to the sample. Vortex at maxi speed for 30s to mix thoroughly.
4. Incubate at 60°C for 30 min.
5. Add ■ 450 µl ACB Buffer, ● 900 µl ACB Buffer, or ☒ 1.8 ml Buffer ACB. Vortex at maxi speed for 30s to mix thoroughly.
6. Incubate the mixture on ice for 5 min.
7. Assemble an HiBind<sup>®</sup> 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 assemble 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 from step 12, centrifuge at maximum speed (13,000 x g) for 5 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 20-150 µl of preheated (60°C) Nuclease-Free Water. Allow the columns to sit for 5 min at room temperature.

15. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Store the eluted DNA at -20°C.