## Contents



## Introduction

E.Z.N.A.® Viral DNA Kit provides a rapid and easy method for the isolation of viral 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 20 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® Viral DNA method is ready for applications such as PCR, viral detection, and genotyping.

E.Z.N.A.® Viral 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 viral 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.® Viral DNA Kit should be stored at 22ºC-25ºC OB Protease should be stored at 15ºC-25°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 BL. In case of such an event, heat the bottle at 37ºC to dissolve. Store Buffer BL at room temperature.

**Expiration Date:** All E.Z.N.A.® Viral 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 30 µg DNA.

## Kit Contents

| Product No. | D3892-02 | D3892-03 | D3892-04 |
|---|---|---|---|
| Purification times | 200 Preps | 500 Preps | 1000 Preps |
| HiBind® DNA Mini Columns | 200 | 500 | 1000 |
| 2 ml Collection Tubes | 600 | 1500 | 3000 |
| Buffer BL | 60 ml | 150 ml | 2 x 150 ml |
| HBC Buffer | 80 ml | 2 x 100 ml | 4 x 100 ml |
| Linear Acrylamide (5mg/ml) | 900 µl | 2 x 1.2 ml | 4.5 ml |
| DNA Wash Buffer Concentrate | 3 x 20 ml | 3 x 50 ml | 6 x 50 ml |
| Elution Buffer | 160 ml | 2 x 200 ml | 4 x 200 ml |
| OB Protease | 2 x 1.2 ml | 4 x1.5 ml | 8 x 1.5 ml |
| User Manual | 1 | 1 | 1 |

## Before Starting

| | | | |
|---|---|---|---|
| **IMPORTANT** | 1 | **Dilute HBC Buffer with Isopropanol as follows and store at room temperature.** | |
| | | D3892-02 | Add 32 ml Isopropanol |
| | | D3892-03 | Add 40 ml Isopropanol per bottle |
| | | D3892-04 | Add 40 ml Isopropanol per bottle |
| | 2 | **Dilute DNA Wash Buffer with 96-100% ethanol as follows and store at room temperature.** | |
| | | D3892-02 | Add 80 ml absolute ethanol per bottle |
| | | D3892-03 | Add 200 ml absolute ethanol per bottle |
| | | D3892-04 | Add 200 ml absolute ethanol per bottle |

**All centrifugation steps must be carried out at room temperature.**



## A. Spin Protocol: Purification of Viral DNA from Plasma or Serum

### Materials and equipments Supplied by User

- **Tabletop microcentrifuge and sterile 1.5 ml tubes.**
- **Water bath -** set to 65°C.
- **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 from1 to 250 µl 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 viral RNA from serum or other non-cellular body fluids use **E.Z.N.A.® Viral RNA Kit**.

Bring samples and OB Protease solution to room temperature and have a water bath equilibrated to 65°C. Preheat an aliquot of Elution Buffer (approximately 0.5 ml per sample) at 65°C. **Carry out all centrifugation steps at room temperature**.

1. Add sample to a sterile microcentrifuge tube and bring the volume up to 250 µl with 10 mM Tris-HCl, PBS, or Elution Buffer provided.

2. Add 10 µl OB Protease and 250 µl of Buffer BL. Add 4µl of Linear Acrylamide to 250 µl Buffer BL. Vortex at maxi speed for 15s to mix thoroughly. If RNA-free genomic DNA is required, add 2µl RNase A (50mg/ml) to each sample.

3. Incubate sample at 65°C for 10 min.

4. Briefly vortex the tube once during incubation.

5. Add 260 µl of absolute ethanol (room temperature, 96-100%) to lysate and vortex at maxi speed for 20s to mix thoroughly. Briefly centrifuge the tube to collect any drops from the inside of the lid.

6. Assemble an HiBind® DNA Mini column in a 2 ml collection tube (provided). Transfer the lysate from step 5 into the column and centrifuge at 8,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.

7. Place the column into a **second 2 ml tube** (provided) and wash by pipetting 500 µl of HBC Buffer. Centrifuge at 8,000 x g for 1 min. Again, Discard flow-through liquid and reuse the collection tube for next step.

8. Place the column into a **same 2 ml tube** from step 7 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.**

9. 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.

10. Place the empty column into the same 2 ml collection tube form step 9, centrifuge at maximum speed (15,000 x g) for 2 min to dry the column. **This step is crucial for ensuring optimal elution in the following step**.

11. Place the column into a sterile 1.5 ml microfuge tube and add 50-100 µl of preheated (65°C) Elution Buffer. Allow tubes to sit for 5 min at room temperature.

12. 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.

    **Note:** Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elution generally give >90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 15 µl to 50 µl Elution Buffer. Volumes lower than 15 µl greatly reduce yields. Alternatively use the first eluate to perform the second elution.

    If necessary the DNA can be concentrated. Add sodium chloride to a final concentration of 0.1 M followed by 2 x volume of absolute (100%) ethanol. Mix well and incubate at -20°C for 10 min. Centrifuge at 10,000 x g for 15 min and



discard supernatant. Add 700 µl of 80% ethanol and centrifuge at 10,000 x g for 2 min. Discard supernatant, air dry the pellet (2 min) and resuspend DNA in 20 µl sterile deionized water or 10 mM Tris-HCl, pH 8.

## B. Vacuum Protocol: Purification of Viral 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 65°C
- **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 1-5 of Protocol A, Spin protocol on page 4.

2. Insert the HiBind® DNA Mini 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. Turn off the vacuum.

   **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 500 µl of HBC Buffer 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 750 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 11-12 on page 5.

## 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 large | If using more than 250 µl of samples, increase volumes of OB Protease/Proteinase K, Buffer BL, and absolute ethanol. Pass aliquots of lysate through one column successively. |
| | 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 5). Incubation of column at 70°C for 5 min with Elution Buffer may increase yields. |



| Problem | Possible Cause | Suggestions |
|---|---|---|
| | Improper washing | Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use. |
| 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. |
| | Poor cell lysis due to incomplete mixing with Buffer BL | Repeat the procedure, this time making sere to vortex the sample with Buffer BL immediately and completely. |
| | Hemoglobin remains on column | After application of sample to column, wash once with 300 µl Buffer BL. |
| No DNA eluted | Poor cell lysis due to improper mixing with Buffer BL. | Mix thoroughly with Buffer BL prior to loading HiBind® column. |
| No DNA eluted | Absolute ethanol not added to Buffer BL. | Before applying sample to column, an aliquot of Buffer BL/ethanol must be added. See protocol above. |
| | 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 BL. | 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. |
| Eluted material has red/brown color | Sample volume too large. | Reduce sample volume and follow directions |
| | Hemoglobin remains on column. | After applying sample, wash column once with 300 µl Buffer BL. |