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Introduction

The E.Z.N.A.® Forensic DNA Kit is designed to provide a rapid and easy method for the isolation of genomic DNA from forensic samples such as dry blood, buccal swabs, and sperm for consistent PCR and Southern analysis. This kit can also be used for the preparation of genomic DNA from mouse tail snips, whole blood, buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps such as precipitation with isopropanol or ethanol are eliminated. DNA purified using the E.Z.N.A.® Forensic DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

The E.Z.N.A.® Forensic DNA Kit is specially designed to work with the OB Specimen Collection Paper (product# OSP-01 & OSP-02) for isolation of genomic DNA from forensic samples such as dry blood and sperm . This kit can be also used for fresh or frozen tissue samples or mouse tail snips (call customer service for detailed protocol).

Principle

E.Z.N.A.® Forensic DNA Kit uses the reversible binding properties of the HiBind® matrix, a new silica-based material, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows genomic DNA up to 50 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® spin columns to which the 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. Each HiBind® column can bind approximately 100 µg DNA. Use of more than 30 mg tissue or 10^7 cells is not recommended.

Storage and Stability

All components of the E.Z.N.A.® Forensic DNA Kit, except the OB Protease, can be stored at 22°C-25°C. Once reconstituted in water, OB Protease must be stored at -20°C. Under these conditions, performance of all components of the kit are guaranteed at least 18 months . Under cool ambient conditions, a precipitate may form in the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve the precipitate. Store Buffer BL at room temperature.

Kit Contents

Product Number	D3591-00	D3591-01	D3591-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA Minicolumns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
Buffer STL	5 ml	20 ml	50 ml
Buffer HB	5 ml	30 ml	110 ml
DNA Wash Buffer	5 ml	20 ml	3 x 20 ml
Elution Buffer	2 ml	30 ml	2 x 50 ml
OB Protease	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

Note: The E.Z.N.A.® Forensic DNA Kit is supplied with enough buffer for the standard protocol. However, due to increased volumes called for in some protocols (such as the buccal swab protocol), fewer preparations may be performed. Also, additional buffers can be purchased separately from Omega Bio-Tek. See the **Accessories** section in the catalog or call customer service for price information.

Before Starting

IMPORTANT

1 Reconstitute OB Protease in 150 µl (Trial Kit) or 1.5 ml (50 and 200 preps) 10 mM Tris-HCl, pH 8. Vortex vial briefly prior to use.

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

D3591-00 Add 20 ml absolute ethanol

D3591-01 Add 80 ml absolute ethanol

D3591-02 Add 80 ml absolute ethanol / bottle

All centrifugation steps must be performed at room temperature.

Standard Protocol For Isolation of DNA From Dried Blood, Body Fluids and Sperm Spots

Dried **blood, body fluids, and sperm** samples on filter paper can be processed using the following method. We recommend using OB Specimen Paper (OSP-01 and OSP-02) for spotting blood, as this unique filter paper disintegrates when incubated in aqueous buffers, allowing for the efficient recovery of DNA. This kit can also be used for samples collected by using other specimen collection papers.

1. Cut or punch out the blood spot (or other sample) from the filter paper. (Up to 200 µl of blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube.

   Note: Use 3-4 punched cycles (3mm diameter) for each DNA isolation.

2. Add 200 µl Buffer STL and incubate at 55°C for 15 minutes. Vortex every 2 min to mix.

3. Add 25 µl OB protease solution and mix by votexing. Incubate for 45 minutes at 60°C with occasional mixing. Briefly centrifuge to remove any droplets from inside the lid.

4. Add 225 µl Buffer BL and votex to mix. Incubate at 60°C for 10 minutes. Briefly centrifuge to remove any droplets from inside the lid.

5. **Add 225 µl absolute ethanol and mix thoroughly by vortexing. Briefly centrifuge to remove any droplets from inside the lid.**

6. Insert each HiBind® DNA Minicolumn into a 2 ml collection tube (provided). Transfer the entire sample from Step 5 into the column, including any precipitate that may have formed. Centrifuge at 8,000 x g for 1 min to bind DNA. Discard collection tube and flow-through liquid.

7. Place each column into a **second 2 ml tube** and wash by pipetting 500 µl of Buffer HB into column. Centrifuge at 8,000 x g for 1 min. Dispose of flow-through liquid and re-use the collection tube.

8. Place each column into a **same 2 ml tube** from step 7 and wash by pipetting 700 µl of DNA Wash Buffer diluted with ethanol into column. Centrifuge at 8,000 x g for 1 min. Dispose of collection tube and flow-through liquid.

   Note: DNA Wash Buffer is provided as a concentrate and must be diluted

with absolute ethanol as indicated on the bottle label and Page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use. Refrigeration is NOT recommended.

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

10. Using the same 2 ml collection tube, centrifuge at maximum speed (>10,000 x g) for 2 minutes to dry the column. **This step is *critical* for removal of residual ethanol that might otherwise interfere with downstream applications.**

11. Place the column into a nuclease-free 1.5 ml microfuge tube and add 50-100 µl of Elution Buffer preheated to 70°C. Allow the tube to sit for 3 minutes at room temperature.

12. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat the elution with a second volume of 50-100 µl Elution Buffer.

Note: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yield per elution. Alternatively, use of the first eluate for second elution will increase DNA concentration.

Blood spots from finger pricks usually contain no more than 50 µl blood and yield approximately 500 ng to 1 µg DNA. This is sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µl preheated Elution Buffer or TE and repeat with the first eluate.

Protocol For Isolation of Genomic DNA From Sperm:

This protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must to be thawed thoroughly before use. Note that lysis time will vary depending on the size and density of the source material.

Make Buffer RS before starting

20 mM Tris·Cl (pH 8.0)
20 mM EDTA
200 mM NaCl
80 mM DTT
4% SDS

DTT oxidizes quickly in aqueous solutions and should also be added just before use. Store the DTT stock solution (1 M) at -20°C.

1. **Add 1-100 µl of sperm to a 1.5 ml microcentrifuge tube. Bring the volume up to 250 µl with Elution Buffer.**

2. **Add 100 µl Buffer RS and 25 µl OB Protease. Vortex to mix and incubate at 55°C in a shaking waterbath to effect complete lysis**. If no shaking waterbath is available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of tissue, but is usually under 1 hours.

3. Add 200 µl Buffer BL and 210 µl absolute ethanol to the sample and mix by vortexing.

4. Follow the standard E.Z.N.A.® forensic DNA protocol from Step 6 on page 4, (i.e apply sample to the HiBind® DNA Mini column).

Protocol For Isolation of Genomic DNA From Buccal Swabs:

This protocol has been tested for the following swab types: cotton, C.E.P. (Life Science). Typical yields from these swabs are 0.5 - 3 µg DNA.

1. Scrape the swabs firmly against the inside of each cheek 6 -7 times. Air or vacuum dry the swabs for 2 hours after collection. The person providing the sample should not eat or drink for at least 30 minutes prior to the sample collection.

2. Separate the swab from the stick. Place the buccal swab into a 2.0 mL microcentrifuge tube and add 550 µl PBS to the tube.

3. Add 25 µl OB protease solution and 550 µl Buffer BL to the sample. Mix immediately by votexing for 30 seconds. Incubate 30 min at 60°C with occasional mixing. Briefly centrifuge to remove any droplets from inside the lid.

4. **Add 550 µl absolute ethanol and mix thoroughly by vortexing. Briefly centrifuge to remove any droplets from inside the lid.**

5. Insert the HiBind® DNA Minicolumn into a 2 ml collection tube (provided). Carefully apply 600 µl of the mixture from Step 4 into the column. Centrifuge at 8,000 x g for 1 min to bind DNA. Discard flow-through liquid and reuse the collection tube for the next step..

6. Insert the column into a new 2 ml collection tube. Carefully apply remaining volume (about 500 µl) of the mixture from Step 4 into the column. Centrifuge at 8,000 x g for 1 min to bind DNA. Discard the flow-through liquid.

7. Follow the standard E.Z.N.A.® forensic DNA protocol from Step 7 on page 4, (i.e apply sample to the HiBind® DNA spin column).

   Note: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yield per elution. Alternatively, use of the first eluate for second elution will increase DNA concentration.

Protocol for Isolation of Bacterial DNA From Biological Fluids:

1. Pellet bacteria by centrifuging 10 minutes at 8,000rpm.

2. Resuspend bacterial pellet with 200 µl STL buffer.

3. Follow the standard protocol (Page 4) from Step 3.

Protocol For Isolation of Genomic DNA From Eye, Nasal, And Other Swabs:

1. Collect the sample and put into 2 ml PBS. Incubate 2-3 hours at 30°C.

2. Pellet bacteria by centrifuging 10 minutes at 8,000rpm.

3. Resuspend bacterial pellet with 200 µl STL buffer.

4. Follow the standard protocol (Page 4) from Step 3.

Protocol For isolation of Genomic DNA from other Forensic Sample

1. Collect the sample and put into tube.

2. Resuspend sample with 200 µl STL buffer.

3. Add 25 µl OB protease solution and mix by votexing. **Incubate at 55°C in a shaking waterbath to effect complete lysis**. If no shaking waterbath is available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of samples, but is usually under 3 hours. One can allow lysis to proceed overnight.

4. Centrifuge at 15,000 x g for 5 min and transfer the supernatant into a new tube.

5. Follow the standard protocol (Page 4) from Step 4.

Forensic DNA Kit Vacuum/Spin Protocol

Note: Please read through previous sections of this manual before using this protocol.

1. **Prepare samples by following the standard protocol in previous sections (Steps 1-5).**

2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.

3. Load the sample/Buffer BL/ethanol mixture into the column.

4. Switch on vacuum source to draw the sample through the column; then turn off the vacuum.

5. Wash the column by adding 500 µl Buffer HB. Draw Buffer HB through the column by turning on the vacuum source.

6. Wash the column by adding 700 µl DNA Wash Buffer. Draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 700 µl DNA wash buffer.

7. Insert the column in a 2 ml collection tube. Then centrifuge 1 minute to dry the column. Drying the column is critical for removal of residual ethanol that might otherwise interfere with downstream applications.

8. Place the column in a nuclease-free 1.5 ml microcentrifuge tube and add 30-50µl TE or water. Allow to stand for 1-2 minutes, then centrifuge 1 minute to elute 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. DNA concentration is calculated 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.

Expected yields vary with both amount, and type of tissue used. 30 mg of fresh tissue will yield 10-40 µg DNA with two elution (each 200 µl).

Troubleshooting Guide

Use the table below to find solutions to any problems you may have with the E.Z.N.A.® Forensic DNA Isolation Kit:

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Extend incubation time of lysis with Buffer STL and protease. 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 30 mg tissue, increase volumes of OB Protease or Proteinase K, Buffer STL, Buffer BL, and 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 sample release from collection paper	Incubate the OB specimen collection paper longer in STL buffer. Shake the tubes frequently.
	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 4 before use.

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.
	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.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer STL and protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein.	After applying to column, wash with 300 µl of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading HiBind® column.
	Poor cell and/or protein lysis in Buffer STL.	Tissue sample must be cut or minced into small pieces. Increase incubation time at 65°C with Buffer STL to ensure that tissue is completely lysed.
	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 vortexed thoroughly.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.

If the above suggestions fail to resolve any problems you are having with the E.Z.N.A.® Forensic DNA Isolation Kit, please feel free to contact our technical specialists at:

Tel: 800.832.8896 (toll-free) 770.931.8400 (local/international)

Fax: 888.624.1688 (toll-free) 770.931.0230 (local/international)

Or direct your questions via e-mail to **info@omegabiotek.com.**

Orders can be placed via telephone, facsimile or e-mail.

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