Contents

Introduction 2

Principle 2

Storage and Stability 2

Binding Capacity 2

Kit Contents 3

Before Starting 3

E.Z.N.A.® Protocol for Tissue 4

Protocol For Cultured Cells 5

Modified Protocol For Mouse Tails Snips 6

E.Z.N.A.® Protocol for Paraffin-Embedded Tissue 8

Vacuum/Spin Protocol 9

Determination of Yield and Quality 9

Troubleshooting Guide 10

Ordering Information 12

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Introduction

The E.Z.N.A.® Tissue DNA Kit provides a rapid and easy method for the isolation of genomic DNA for consistent PCR and Southern analyses. Up to 30 mg tissue or up to 1 cm sections of mouse tail can be readily processed in one prep. Th method can also be used for preparation of genomic DNA from mouse tail snips, cultured cells, 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.® Tissue DNA method is ready for applications such as PCR, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.® Tissue DNA Kits use the reversible binding properties of the HiBind® matrix, a new silica-based material, combined with the speed of mini-column spin technology. A specially formulated buffer system allows genomic DNA up to 60 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® 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.® Tissue DNA Kit, except the OB Protease can be stored at 22°C-25°C and are guaranteed for at least 24 months from the date of purchase. Once reconstituted in water, OB Protease must be stored at -20°C. 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. Store Buffer BL at room temperature.

New in this edition

- New capped S-Spin columns (D3395 & D3396) ensure the elimination of potential contamination during operation.
- Optional vacuum-spin (for V-Spin columns) protocol included(Page 9). (V-Spin columns are non-standard for this kit and must be specified at time of order.)

Binding Capacity

Each HiBind®column can bind up to 100 µg DNA. Use of more than 30 mg tissue or 10^7 cells is not recommended.

Kit Contents

Product	D3495-00 D3496-00 D3395-00 D3396-00	D3495-01 D3496-01 D3395-01 D3396-01	D3495-02 D3496-02 D3395-02 D3396-02
Components			
HiBind® DNA columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
Buffer TL	5 ml	20 ml	50 ml
Wash Buffer Concentrate	12 ml	2 x 24 ml	3 x 40 ml
Elution Buffer	2 ml	30 ml	2 x 50 ml
OB Protease (D3496 &D3396)	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

Buffer BL contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Before Starting

IMPORTANT

1 **D3496**: 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.
D3495& D3395: Prepare a Proteinase K (molecular biology grade) stock solution at 20 mg/ml in Tris HCl buffer.
We recommend that you aliquot and store vials of reconstituted protease at -20°C.

2 Wash Buffer Concentrate must be diluted with absolute ethanol as follows:

D3495-00 & D3496-00 **D3395-00 & D3396-00**	Add 18 ml ethanol
D3495-01 & D3496-01 **D3395-01 & D3396-01**	Add 36 ml ethanol / bottle
D3495-02 & D3496-02 **D3395-02 & D3396-02**	Add 60 ml ethanol / bottle

Note: *All centrifugation steps must be performed at room temperature.

E.Z.N.A.® Protocol for Tissue

Have the following reagents and supplies ready before starting procedure:

- Tabletop microcentrifuge and nuclease-free1.5 ml tubes.
- Warm up Elution Buffer (0.5 ml per sample) to 70°C.
- Have a shaking waterbath set to 55°C.
- Absolute ethanol - approximately 0.3 ml per sample.
- Proteinase K - stock solution at 20 mg/ml (for D3495/D3395).

This method allows genomic DNA isolation from up to 30 mg tissue. Yields vary depending on source.

OPTIONAL: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue to a clean 1.5 ml tube. Add 200 µl Buffer TL and proceed to step 2 below.

1. **Mince up to 30 mg of tissue and place into a 1.5 ml microfuge tube. Add 200 µl Buffer TL.** Cut the tissue into small pieces to speed up lysis. For samples larger than 30 mg, simply scale up the volume of Buffer TL used; for a 60 mg sample use 400 µl buffer.

2. **Add 25 µl of OB Protease (D3496& D3396) or Proteinase K (D3495& D3395) at 20 mg/ml solution, vortex to mix well, 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 3 hours. One can allow lysis to proceed overnight.

   The volume of OB Protease (or proteinase K) used will need to be adjusted based on amount of starting material; use 50 µl for a 60 mg tissue sample.

3. **OPTIONAL:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 20 µl (assuming a sample size of 30 mg) RNase A (25 mg/ml) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol.

4. **Add 220 µl Buffer BL and vortex to mix. Incubate at 70°C for 10 minutes.** A wispy precipitate may form on addition of Buffer BL, but does not interfere with DNA recovery. Adjust the volume of Buffer BL required based on amount of starting material.

5. **Add 220 µl absolute ethanol and mix thoroughly by vortexing.** Adjust the volume of ethanol if greater than 30 mg tissue is used).

6. Assemble a HiBind® spin column in 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 the collection tube and flow-through liquid.

7. Place the column into a **second 2 ml tube** and wash by pipetting in 650 µl of Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

8. Using a **new collection tube**, wash the column with a second 650 µl of Wash Buffer and centrifuge as above. Discard flow-through.

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

10. Place the column into a nublease-free 1.5 ml microfuge tube and add 200 µl of preheated (70°C) Elution Buffer. Allow tubes to sit for 3 min at room temperature.

11. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat the elution with a second 200 µl of Elution Buffer.

Incubation at 70°C rather than at room temperature will give a modest increase in DNA yield per elution. Alternatively the second elution may be performed using the first eluate. The expected yield from a 30 mg sample is 8-35 µg genomic DNA, depending on type of tissue.

Protocol For Isolation of Genomic DNA From Cultured Cells:

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

1. Prepare the cell suspension
   - 1a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation, wash the cells with PBS and resuspend cells with 200µl cold (4°C) PBS. Proceed with step 2 of this protocol.
   - 1b. For cells grown in suspension, pellet 5 x 10^6 cells by spinning at 1200 x g in a centrifuge tube. Discard the supernatant, and wash the cells once with PBS, and resuspend cells with 200µl cold (4°C) PBS.
   - 1c. For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or scrape with rubber policemen. Wash cells twice, and resuspend the cells with 200µl cold (4°C) PBS.

2. **Add 25 µl of OB Protease (D3496/D3396) or Proteinase K (D3495/D3395) at 20 mg/ml solution, vortex to mix well, and incubate at 65°C in a waterbath for 5 minutes to effect complete lysis**.

3. **Add 220 µl Buffer BL and vortex to mix. Incubate at 70°C for 10 minutes.** A wispy precipitate may form on addition of Buffer BL; it does not interfere with DNA recovery. Adjust the volume of Buffer BL required based on amount of starting material.

4. **Add 220 µl absolute ethanol and mix thoroughly by vortexing.**

5. Assemble a HiBind® spin column in 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 the collection tube and flow-through liquid.

6. Place the column into a **second 2 ml tube** and wash by pipetting 650 µl of Wash Buffer diluted with ethanol. Centrifuge at 8,000 x g for 1 min. Again, dispose of collection tube and flow-through liquid.

7. Using a **new collection tube**, wash the column with a second 650 µl of Wash Buffer and centrifuge as above. Discard flow-through.

8. Using the same 2ml collection tube, centrifuge empty column at maximum speed (10,000 x g) for 2 min to dry the HiBind® membrane. **This step is crucial for ensuring optimal elution in the following step**.

9. Place the column into a sterile 1.5 ml microfuge tube and add 200 µl of preheated (70°C) Elution Buffer. Allow tubes to sit for 3 min at room temperature.

10. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat the elution with a second 200 µl of Elution Buffer.

Modified Protocol For Mouse Tails Snips

Before starting have the following ready:

- Tabletop microcentrifuge and sterile 1.5 ml tubes.
- Shaking waterbath set to 55°C.
- Elution Buffer (~0.5 ml per sample) equilibrated to 70° C.
- **For each sample, premix 200 µl Buffer BL with 210 µl absolute ethanol and vortex. This can be prepared fresh, or pre-made and stored at room temperature. Do not store this mixture for more than 1 month.**
- Prepare OB protease or Proteinase K as instructed on Page 3 of main Tissue DNA protocol. Reconstitute each vial 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. We recommend that you aliquot and store vials of protease at -20°C.

Bring frozen samples and OB Protease or Proteinase K solution to room temperature and, preheat an aliquot of Elution Buffer (approximately 0.5 ml per sample) at 65°C.

1. **Snip two pieces of mouse tail 0.2 - 0.5 cm in length, place into a nuclease-free 1.5 ml microcentrifuge tube, and add 180 µl of Buffer TL**. If necessary cauterize the wound to stop bleeding. Having appropriately earmarked the animal, return it to a clean cage.

Note: Mice should not be older that 6 weeks, since lysis will be more difficult in older animals, resulting in suboptimal DNA yields. If possible, obtain tail biopsy at 2-4 weeks and freeze samples at - 70°C until DNA is extracted.

Add 25 µl of OB protease (D3496) or Proteinase K at 20 mg/ml (D3495), vortex to mix and incubate in a 55°C shaking waterbath for 1-4 hours or until lysis is complete. If no shaking waterbath is available, vortex vigorously every 20-30 minutes. Incomplete lysis may block column flow and significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail and age of animal; 0.5 cm tail pieces from 2 week-old mice typically lyse in approximately 2 hours. For older animals an overnight incubation may improve yields. Note that bone and hair will not lyse.

2. **Centrifuge for 5 minutes at 10,000 x g to pellet insoluble tissue debris and hair**. Carefully aspirate the supernatant and transfer to a sterile microfuge tube leaving behind any insoluble pellet.

   OPTIONAL: Mouse tail tissue contains RNA that can co-purify with the DNA. This will not interfere with PCR reactions, but other enzymatic reactions may be affected. To remove RNA, add 15 µl of RNase A (25 mg/ml) and incubate 2 minutes at room temperature.

3. **Add ONE volune BL followed by ONE volume isopropanol. Vortex thoroughly to mix.** *Thorough mixing is essential at this point*.

4. **Transfer the entire sample (including any wispy precipitate that may form) to a HiBind® DNA column (provided) assembled in a 2 ml collection tube (also provided).** Centrifuge the assembly at 8,000 x g for 1 minute to bind DNA. Discard the collection tube and filtrate.

5. **Place the column into a second 2 ml tube and wash by pipetting 650 µl of Wash Buffer diluted with ethanol.** Centrifuge at 8,000 x g for 1 min. Again dispose of collection tube and flow-through.

6. **Using a new collection tube, wash the column with a second 650 µl of Wash Buffer and centrifuge as above.** Discard flow-through.

7. **Centrifuge at maximum speed for 2 min to dry the column**. **This step is crucial for ensuring optimal elution in the following step**.

8. Place the column into a nuclease-free 1.5 ml microfuge tube and add 200 µl of preheated (70°C) Elution Buffer. Allow tubes to sit for 3 min at room temperature. Incubation at 60°C to 70°C during this period may increase yields.

9. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat elution step with an additional 200 µl of preheated Elution Buffer. Alternatively, a second elution using the first eluate may give higher final concentration. Discard column.

Note: Each 200 µl elution typically yields 60-70% of the DNA bound to the column. Thus two elutions 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 50 µl to 100 µl Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µl greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon addition of Elution Buffer. Alternatively, a second elution can be done using first eluate.

If necessary the DNA can be concentrated. Add sodium chloride to a final concentration of 0.1 M followed by 2X 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.

Protocol for Paraffin-Embedded Tissue

1. Place not more than 30 mg tissue (2-5 of ~20 µm sections) in a clean 2 ml microfuge tube.

2. Extract the sample with 1 ml xylene to remove the paraffin. Incubate at room temperature for 30 minutes. Mix thoroughly by vortexing.

3. Centrifuge the tube at 10,000 x g for 10 min at room temperature. Discard supernatant without disturbing the tissue pellet.

4. Rinse the pellet with 1 ml absolute ethanol to remove traces of xylene, incubate at room temperature for 10 minutes. Centrifuge at 10,000 x g for 5 min at room temperature. Discard the ethanol without disturbing the tissue pellet.

5. Repeat step 4 with 75% ethanol rinse.

6. Air dry tissue pellet at 37°C for 15 min.

7. Add 200 µl Buffer TL to the tissue and follow the main Tissue DNA Protocol from step 2 using either OB protease or Proteinase K (page 4).

For elution of DNA, we recommend using 100 µl of Elution Buffer warmed to 70 °C. Yields will depend on size and age of sample. Certain samples may require prolonged lysis with Buffer TL.

Note: Tissue fixed with paraformaldehyde will yield degraded DNA or RNA. The extent of degradation depends on type of fixative used, but the size of DNA obtained is usually less than 500 bp. Degradation is not caused by the E.Z.N.A.® Tissue DNA protocol, and for PCR detection of segments smaller than 500 bp satisfactory results can be obtained.

Vacuum/Spin Protocol (V-Spin column only)

Carry out homogenization, Protease digestion, and loading onto HiBind® DNA column as indicated in previous protocols above. Instead of continuing with centrifugation, follow steps below.

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

1. Prepare the vacuum manifold according to manufacturer' s instructions and place the HiBind® DNA V-Spin column in the manifold.
2. Load the sample into a HiBind® DNA V-spin column.
3. Switch on vacuum source to draw the sample through the column, and then turn off the vacuum.
4. Wash the column by adding 750 µl DNA wash buffer and draw the wash buffer through the column by turning on the vacuum source.
5. Wash the column again by adding 750 µl DNA wash buffer and draw the wash buffer through the column by turning on the vacuum source.
6. Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 50-100µl DNA elution buffer. Allow to stand for 1-2 minute, 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:

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

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 elutions (each 200 µl).

Troubleshooting Guide

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

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Extend incubation time of lysis with Buffer TL 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 TL, 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 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.
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 Tland protease. Ensure that no visible pieces of tissue remain.

Problem	Possible Cause	Suggestions
	Samples are rich in protein.	AaaAfter 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 TL.	Tissue sample must be cut or minced into small pieces. Increase incubation time at 65°C with Buffer TL 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.

NOTES