

Contents

Introduction.	2
Principle.	2
Storage and Stability.	2
Binding Capacity.	2
Kit Contents.	3
Before Starting.	3
Isolation of DNA from Tissue.	4
Protocol for smaller volume of blood, serum or body fluids.	5
Protocol for dried blood, body fluids and sperm spot.	6
Protocol for swabs.	8
Protocol fo forensic samples.	9
Protocol for Paraffin-embedded tissue.	10
Troubleshooting Guide.	11

Introduction

The E.Z.N.A.™ MicroElute Genomic DNA Kit provides a rapid and easy method for the isolation of genomic DNA and mitochondrial DNA from small size or a large volume of samples for consistent PCR and other downstream applications. This kit can be used for the isolation of genomic DNA from micro-dissected tissue, cultured cells, blood, dry blood, swabs, buffy coat, serum, urine 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.

Principle

The E.Z.N.A.™ MicroElute Genomic DNA Kit uses the reversible binding properties of the HiBind® matrix, a new silica-based material, combined with the MicroElute column spin technology which allows smaller elution volume as little as 10 µL. A specially formulated buffer system allows genomic DNA up to 40kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® Micro-spin columns to bind DNA, 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.™ MicroElute Genomic 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 aliquoted and 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.

Binding Capacity

Each HiBind® MicroElute column can bind up to 30 µg genomic DNA. Use of more than 10mg tissue or 5 x 10⁶ cells is not recommended.

Kit Contents

Product	D3096-00	D3096-01	D3096-02
2 mL Collection Tubes	10	100	400
Buffer BL	5 mL	35 mL	125
Buffer TL	5 mL	35 mL	125 mL
Buffer HB	3 mL	30 mL	120 mL
DNA Wash Buffer Concentrate	4 mL	40 mL	3 x 40 mL
Linear Acrylamide (5mg/mL)	25 µl	250 µl	900 µL
Equilibration Buffer	1.5 mL	7 mL	25 mL
Elution Buffer	2 mL	10 mL	40 mL
OB Protease	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

* Buffer BL contains a chaotropic salt. Equilibration Buffer contains Sodium Hydroxide. Use gloves and protective eyewear when handling this solution.

Before Starting

IMPORTANT	Reconstitute OB Protease with Elution Buffer , aliquot and store the solution at -20 °C
	D3096-00 Dissolve in 150µL
	D3096-01 Dissolve in 1.50mL
	D3096-02 Dissolve in 1.50mL per tube
	Dilute DNA Wash Buffer must be diluted with absolute ethanol (96-100%)
	D3096-00 Add 6mL absolute ethanol(96-100%)
	D3096-01 Add 60mL absolute ethanol(96-100%)
	D3096-02 Add 60mL absolute ethanol per bottom
	Linear Acrylamide: To purify very small amounts of DNA from a sample, such as low volumes of blood (<10ul) or micro-dissected tissues, we recommend adding Linear Acrylamide to Buffer BL to enhance the DNA binding ability of the column. In most cases, adding 5-10ug (1-2ul) per sample is sufficient.

Isolation of DNA from Tissue

Equipment to be supplied by user

- Absolute ethanol (96-100%)
- 1.5ml or 2mL microcentrifuge tubes
- Water Bath or heating block preset at 60°C
- Water Bath or heating block preset at 70°C
- Microcentrifuge with rotor for 2mL tubes
- DTT (for processing hair and semen)
- Elution Buffer or ddH₂O pre-warmed at 70°C
- Tabletop centrifuge capable of 13,000x rpm
- **Optional:** Omega Homogenizer Column (HCR-01) for collect any remaining liquid from paper or swab.

The following protocol allows genomic DNA isolation from up to 10mg 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 1.5mL tube. Add 200µL Buffer TL and proceed to step 2 below.

1. Mince up to 10mg of tissue and place into a 1.5mL microfuge tube. Add 200µL Buffer TL. Cut the tissue into small pieces to speed up lysis.
2. Add 20µL OB Protease solution, vortex to mix well, and incubate at 55°C in a shaking waterbath to effect complete lysis. If no shaking water bath 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.
3. Centrifuge at 13,000xg for 2 minutes to pellet any undigested particles. Aspirate or pipette off the supernatant into a clean 1.5ml microfuge tube.
4. **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 5µL (assuming a sample size of 10mg) RNase A (25mg/mL) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol.
5. Add 220µL Buffer BL and vortex to mix well. Incubate at 70°C for 10 minutes. If Linear Acrylamide is needed, add 1µL of Linear Acrylamide to 220µL BL Buffer.
NOTE: Step 7 can be performed during incubation time.
6. Add 220µL absolute ethanol and mix thoroughly by vortexing for 15 seconds at max speed. Briefly centrifuge to bring down any liquid from the top of the lid.

7. Insert a HiBind® DNA Mini Column into a 2 ml collection tube (provided). Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds
8. Assemble a HiBind® MicroElute column in a 2mL collection tube (provided). Transfer the entire solution from Step 6 into the column including any precipitate that may have formed. Centrifuge at 13,000 xg for 30 to 60 seconds to bind DNA and discard the flow-through.
9. Add 500µL of Buffer HB. Close the lid and centrifuge at 13,000xg for 30 to 60 seconds and discard the flow-through.
10. Add 700µL of DNA Wash Buffer diluted with ethanol. Centrifuge at 13,000xg for 30 to 60 seconds and dispose of flow-through.
NOTE: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions or on Page 3 for preparation.

Optional: Repeat step 10

11. Centrifuge the column at 13,000xg for 2 min to dry the HiBind® membrane.
NOTE: This step is crucial for ensuring optimal elution in the following step.
12. Place the column into a nuclease-free 1.5ml microfuge tube (Not supplied). Add 10-50µL of preheated (70°C) Elution Buffer onto the center of membrane. Allow to sit for 3 min at room temperature.
13. To elute DNA from the column, centrifuge at 13,000xg for 1 min.
NOTE: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yields per elution. Alternatively the second elution may be performed using the first eluate or using the second 10-100µL of preheated Elution Buffer.

Isolation of DNA from a Small Volume of Blood, Serum or Fluids

This protocol is designed for the rapid isolation of DNA from 1-100 µL of blood (treated with EDTA, citrate, or heparin-based anticoagulants) or serum, saliva, urine, buffy coat, serum, and plasma.

1. Prepare 1-100µL samples (sample must be at room temperature before beginning) in a 1.5ml microfuge tube.
2. Adjust the sample volume to 100ul using PBS Buffer.
3. Add 20µL of Protease solution and mix well by vortexing.
4. Add 120µL Buffer BL and vortex to mix. Incubate at 70°C for 10 minutes.
NOTE: If the blood volume is less than 10µl, the addition of 1µl of linear acrylamide to each sample is recommended.
5. Add 120µL absolute ethanol and mix thoroughly by vortexing for 15 seconds at maximum speed. Briefly centrifuge to bring down any liquid from the top of the lid.
6. Insert a HiBind® DNA Mini Column into a 2 ml collection tube (provided). Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds
7. Assemble a HiBind® MicroElute column in a 2ml collection tube (provided). Transfer the entire solution from Step 5 into the column, including any precipitate that may have formed. Centrifuge at 13,000xg for 30 to 60 seconds to bind the DNA and discard the flow-through.
8. Add 500µl of Buffer HB. Centrifuge at 13,000xg for 30 to 60 seconds and discard the flow-through.
9. Add 700µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 13,000xg for 30 to 60 seconds and discard the flow-through.
NOTE: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle or Page 3 for preparation.
- 10. OPTIONAL: REPEAT step 9.**
11. Centrifuge the empty column at 13,000x g for 2min to dry the HiBind® membrane.
NOTE: This step is crucial for ensuring optimal elution in the following step.
12. Place the column into a sterile 1.5ml microfuge tube and add 10-50µl of preheated (70°C) Elution Buffer onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.
13. To elute DNA from the column, centrifuge at 13,000xg for 1 min.
NOTE: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yields per elution. Alternatively the second elution may be performed using the first eluate or using the second 10-50µl of preheated Elution Buffer.

Isolation of DNA from Dried Blood, Fluids, and Semen

Dried blood, body fluids, and semen samples on filter paper can be processed using the following method. We recommend using OBI Specimen Paper (OBP-01 and OBP-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 using other specimen collection papers

Before starting:

Bring frozen samples and OB Protease solution to room temperature, preheat an aliquot of Elution Buffer (approximately 0.5ml per sample) at 70°C.

Procedure:

1. Cut or punch out the blood (or other sample) spot from the filter paper. Tear or cut filter into small pieces and place into a 1.5ml centrifuge tube (not provided).
NOTE: Use 1-3 punched circles (3mm diameter) for each DNA isolation.
2. Add 200µl Buffer TL, followed by 20µl of Reconstituted OB Protease solution. Incubate mixture at 60°C for 45-60 minutes. Mix the samples several times during incubation by vortexing.
3. Briefly centrifuge to bring down any liquid from the top of the tube.
4. Add 220µl Buffer BL, mix thoroughly by vortexing for 20 seconds. If only one punch card is processed, add 1µl of linear acrylamide to the sample.
5. Place the tube in a heating block or waterbath preset at 70°C. Incubate for 10 minutes. Vortex the tube for 10 seconds several times during incubation. Briefly centrifuge to bring down any liquid from the top of the tube.
NOTE: For maximum yield collect any remaining liquid from paper and transfer the entire sample, including paper, into a Homogenizer Column (not supplied) and centrifuge at 13,000x g for 2 minutes to collect all of the lysate. Homogenizer columns can be purchased separately (Product No. HCR-001, HCR-003).
6. Insert a HiBind® DNA Mini Column into a 2 ml collection tube (provided). Add 100µl Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds
7. Add 220ul absolute ethanol and mix thoroughly by vortexing for 20 seconds. Briefly centrifuge to bring down any liquid from the top of the tube.
8. Assemble a HiBind® MicroElute column in a 2ml collection tube (provided). Transfer the entire lysate from Step 7 into the HiBind DNA MicroElute column including any precipitate. Centrifuge at 13,000xg for 30 to 60 seconds to bind DNA and discard flow-through.

9. Add 500µl of Buffer HB. Centrifuge at 13,000x g for 30 to 60 seconds to bind DNA and discard the flow-through.
10. Add 700µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 13,000x g for 30 to 60 seconds to bind DNA and discard the flow-through.
NOTE: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle or Page 3 for preparation.
11. **REPEAT** step 10.
12. Centrifuge the empty column at 13,000x g for 2 min to dry the HiBind® membrane.
NOTE: This step is crucial for ensuring optimal elution in the following step.
13. Place the column into a sterile 1.5ml microfuge tube and add 10-50µl of preheated (70°C) Elution Buffer onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.
14. To elute DNA from the column, centrifuge at 13,000x g for 1 min.
NOTE: Incubation at 70°C rather than at room temperature will give a modest increase in DNA yields per elution. Alternatively the second elution may be performed using the first eluate or using the second 10-50µl of preheated Elution Buffer.

Isolation of Genomic DNA from Cotton Swabs

This protocol is designed for the isolation of genomic DNA from sperm swabs, blood swabs and buccal swabs.

1. Place the swab in a 2ml microcentrifuge tube.
2. Add 600µl Buffer TL and 20µl Proteinase K solution into the tube. Mix thoroughly by vortexing for 30 seconds.
3. Incubate the tube in a heating block or a waterbath at 55°C for at least 1 hour. Mix the sample few times during the incubation by briefly vortexing.
4. Briefly centrifuge to bring down any liquid from the top of the tube.
5. Add 620µl Buffer BL, close the lid, mix thoroughly by vortexing. If Linear Acrylamide is needed, add 1 µL of dissolved Linear Acrylamide to 660 µL BL Buffer. See page 4 for detail instruction.
6. Place the tube in a heating block or waterbath preset at 70°C. Incubate for 10 minutes. Vortex the tube 10 seconds few times during incubation.
7. Briefly centrifuge the centrifuge tube to bring down any liquid drop from inside of the lid.

8. Add 620 μ L absolute ethanol and mix thoroughly by vortexing for 20s at maxi speed. Centrifuge briefly to bring down any liquid from inside of lid.
9. Insert a HiBind® DNA Mini Column into a 2 ml collection tube (provided). Add 100 μ L Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds
10. Assemble a HiBind® MicroElute column in a 2 mL collection tube (provided). Transfer the 700 μ L of lysate from Step 8 into the column including any precipitate that may have formed. Close the lid and centrifuge at 8,000 xg for 1 min to bind DNA. Discard flow-through liquid and re-use the collection tube.
11. Place the HiBind® MicroElute column into same collection tube from step 10 and repeat step 9 until all of remaining lysate from step 8 has pass through the HiBind® MicroElute column. Discard the flow-through and collection tube.

Note: For maximum yield, Collection any remaining liquid from swab, transfer all swab into a Homogenizer Column (not supplied) and centrifuge at 20,000 xg for 2 minutes to collect remaining lysates. Homogenizer column can be purchased separately from Omega Bio-tek (Product No. HCR-001 an HCR-003).

12. Place the column into a new collection tube (supplied). Add 500 μ L of Buffer HB in the column. Close the lid and centrifuge at 8000 xg for 1 minute. Discard the flow-through and collection tube.
13. Place the column into a new 2 mL tube (supplied) and wash by pipetting 650 μ L of DNA Wash Buffer *diluted with ethanol*. Centrifuge at 8,000 xg for 1 min. Discard flow-through liquid and re-use the collection tube.

Note: DNA Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions or on Page 3 for preparation.

14. Using the same collection tube from step 12, wash the column with a second 650 μ L of DNA Wash Buffer *diluted with ethanol* and centrifuge as above. Discard flow-through and re-use the collection tube.
15. Using the same 2 mL collection tube, centrifuge empty column at maximum speed (20,000 xg) for 3 min to dry the HiBind® membrane. *This step is crucial for ensuring optimal elution in the following step.*
16. Place the column into a sterile 1.5 mL microfuge tube and add 10-50 μ L of preheated (70°C) Elution Buffer or water onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.
17. To elute DNA from the column, centrifuge at 20,000 xg for 1 min. 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 or using the second 10-100 μ L of preheated Elution Buffer or water.

Isolation of DNA from Forensic samples

This protocol is designed for isolation of genomic DNA from forensic samples such as hair, cigarette butts, nail clippings, material stained with blood, saliva, or semen stints.

1. Cut the sample to small pieces and place into a 2 mL microcentrifuge tube. Add 300 μ L of Buffer TL into the tube, mix thoroughly by vortexing. If process semen stints, add 20 μ L of DTT for each sample.
2. Add 20 μ L of OB Protease solution to each sample. Incubate sample at 60°C for 45-60 minutes or overnight if necessary. Mix the samples several times during incubation by vortexing.
3. Centrifuge the tube to spin down any liquid drop from inside of the lid and any material that is not lysed.
4. Add 320 μ L Buffer BL, close the lid and mix thoroughly by vortexing for 20 seconds. If only one punch card is processed, add 4 μ L of Linear Acrylamide to the sample. See page 4 for detail instruction.
5. Place the tube in a heating block or waterbath preset at 70°C. Incubate for 10 minutes. Vortex the tube 10 seconds few times during incubation.
6. Centrifuge at 20,000 xg for 5 minutes. Transfer the supernatant to a new 2 mL microcentrifuge tube. Add 0.5 volume of absolute ethanol and mix thoroughly by vortexing for 20s at maxi speed. Centrifuge briefly to bring down any liquid from inside of lid.
7. Insert a HiBind® DNA Mini Column into a 2 ml collection tube (provided). Add 100 μ L Equilibration Buffer into the column. Let the column sit for 4 minutes at room temperature. Spin at maximum speed for 20 seconds
8. Assemble a HiBind® MicroElute column in a 2 mL collection tube (provided). Transfer the 600 μ L of sample from Step 6 into the column including any precipitate that may have formed. Close the lid and centrifuge at 8,000 xg for 1 min to bind DNA. Discard flow-through liquid and re-use the collection tube.
9. Place the HiBind® MicroElute column into the same collection tube from step 8 and transfer the remaining lysate from step 6 into the column. Centrifuge at 8000 xg for 1 minute. Discard the flow-through and collection tube.
10. Place the column into a new collection tube (supplied). Add 500 μ L of Buffer HB in the column. Close the lid and centrifuge at 8000 xg for 1 minute. Discard the flow-through and collection tube.
11. Place the column into a new 2 mL tube (supplied) and wash by pipetting 650 μ L of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 xg for 1 min. Discard the flow-through and re-use the collection tube.

NOTE: DNAWash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions or on Page 3 for preparation.

12. Using a same collection tube from step 10, wash the column with a second 650 μ L of DNAWash Buffer *diluted with ethanol* and centrifuge as above. Discard flow-through and re-use the collection tube.
13. Using the same 2 mL collection tube, centrifuge empty column at maximum speed (20,000 xg) for 3 min to dry the HiBind® membrane. *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 10-50 μ L of preheated (70°C) Elution Buffer or water onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.
15. To elute DNA from the column, centrifuge at 20,000 xg for 1 min.

Isolation of DNA from Paraffin-Embedded Tissue

This protocol is designed for purification of total DNA from fixed, paraffin-Embedded tissue. Please note that the length of DNA purified from fixed tissue is normally <650bp, depending on the age and storage condition of sample.

1. Place the a small piece of (<15 mg) of paraffin-embedded tissue in a 2 mL centrifuge tube (not supplied).
2. Add 1 mL xylene, Mix vigorously by vortexing.
3. Centrifuge at 20, 000 xg for 5 minutes at room temperature.
4. Aspirate the supernatant by pipetting. Do not remove any pellet.
5. Add 1 mL of absolute ethanol (96-100%) to the tube and mix gently by vortexing.
6. Centrifuge at 20, 000 xg for 5 minutes at room temperature.
7. Aspirate the supernatant by pipetting. Do not remove any pellet.
8. Wash the pellet again with 1 mL absolute ethanol by repeating step 6-7.
9. Dry the pellet by air or place it at heat block preset at 37°C for 5 minutes.
10. Resuspend the pellet with 200 μ L TL Buffer and proceed the DNA isolation from step 2 of the protocol " Protocol for Isolating DNA from Small Tissue Sample).

Troubleshooting Guide

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 μ L 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 A260/A280 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 sure 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.
	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 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.

Problem	Possible Cause	Suggestions
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.