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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 volume of samples for consistent PCR and other downstream applications. This kit can be used for preparation 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 10µl. A specially formulated buffer system allows genomic DNA up to 40 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® Mircro-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 10 µg genomic DNA. Use of more than 10 mg tissue or 5 x 10^6 cells is not recommended.

Kit Contents

Product	D3096-00	D3096-01	D3096-02
HiBind® MicroElute Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	35 ml	125 ml
Buffer TL	5 ml	35 ml	125 ml
Buffer HB	3 ml	30 ml	120 ml
DNA Wash Buffer Concentrate	2 ml	20 ml	3 x 20 ml
Linear Acrylamide (5mg/ml)	25µl	250µl	900µl
Elution Buffer	2 ml	10 ml	40 ml
Protease Storage Buffer	200 ul	1.8 ml	7 ml
OB Protease	3 mg	30 mg	4 x 30 mg
User Manual	1	1	1

Before Starting

IMPORTANT

1 Reconstitute OB Protease with Protease Storage Buffer as follows, then aliquot and store the solution at -20°C
D3096-00: Dissolve in 150 µl Protease Storage Buffer
D3096-01: Dissolve in 1.50 ml Protease Storage Buffer
D3096-01: Dissolve in 1.50 ml Protease Storage Buffer per tube

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

D3096-00 Add 8 ml absolute ethanol(96-100%).
D3096-01 Add 80 ml absolute ethanol(96-100%).
D3096-02 Add 80ml absolute ethanol per bottle.

3 Linear Acrylamide: For purification of DNA from very small amounts of samples, such as low volume of blood (<10ul) or micro-dissected tissues, we recommend to add Linear Acrylamide to Buffer BL to enhance DNA binding ability to the column.

Equipment and Reagents to Be Supplied by User

- Absolute ethanol (96-100%)
- 1.5 ml 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 ddH_2O pre-warmed at 70°C
- Tabletop centrifuge capable of 20,000 x g (13,000 x rpm)
- Optional: Omega **Homogenizer Column** (HCR-01) for collect any remaining liquid from paper or swab.

E.Z.N.A.® Protocol for Small Size of Tissue

This method allows genomic DNA isolation from up to 10 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 10 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.

2. **Add 20 µl 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 20,000 x g for 2 minutes to remove any undigested particles from cell lysates.

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 5 µl (assuming a sample size of 10 mg) RNase A (25 mg/ml) and incubate at room temperature for 2 minutes. Proceed with the tissue protocol.

5. **Add 220 µl Buffer BL and vortex to mix well. Incubate at 70°C for 10 minutes.** If **Linear Acrylamide** is needed, add 4µl of Linear Acrylamide to 220µl Buffer BL.

6. **Add 220 µl absolute ethanol and mix thoroughly by vortexing for 15s at maxi speed.** Centrifuge briefly to bring down any liquid from inside of lid.

7. Assemble a HiBind® MicroElute column in a 2 ml collection tube (provided). Transfer the entire solution from Step 6 into the column including any precipitate that may have formed. Close the lid and centrifuge at 8,000 x g for 1 min to bind DNA. Discard the collection tube and flow-through liquid.

8. Place the column into a new collection tube (supplied). **Add 500µl of Buffer HB in the column.** Close the lid and centrifuge at 8000 x g for 1 minute. Discard the flow-through and re-use collection tube.

9. Place the column into **the same 2 ml collection tube** from step 8 and wash by pipetting in 650 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: *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.*

10. Using a **new collection tube**, wash the column with a second 650 µl of DNA Wash Buffer ***diluted with ethanol*** and centrifuge as above. Discard flow-through and re-sue the collection tube.

11. Using the same 2ml collection tube, centrifuge empty column at maximum speed (20,000 x g) for 3 min to dry the HiBind® membrane. ***This step is crucial for ensuring optimal elution in the following step.***

12. Place the column into a nuclease-free 1.5 ml microfuge tube (Not supplied) and add 10-50 µl of preheated (70ºC) Elution Buffer or water onto the center of membrane. Allow tubes to sit for 3 min at room temperature.

13. To elute DNA from the column, centrifuge at 20,000 x g for 1 min.

Note: Incubation at 70ºC 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 10-100µl of preheated Elution Buffer or water.

E.Z.N.A.® Protocol For Smaller volume of Blood, serum or body fuilds

This protocol is designed for the rapid isolation of DNA from 1-100µl of blood treated with EDTA, citrate, or heparin-based anticoagulants. This method can be used for preparation of genomic DNA from serum, saliva, urine, buffy coat, serum, and plasma.

1. Prepare 1-100µl samples (sample must be equilibrated to room temperature before process) into a 1.5 ml microfuge tube.

2. Adjust the sample volume to 100µl with PBS Buffer.

3. **Add 20 µl of Protease solution and mix well by vortexing.**

4. **Add 120 µl Buffer BL and vortex to mix. Incubate at 70ºC for 10 minutes.** If the blood volume is less than 10µl, 4µl of Linear acrylamide is recommended to be added to each sample.

5. **Add 120 µl absolute ethanol and mix thoroughly by vortexing for 15s at maxi speed.** Bring down any liquid drop from inside of lid by brief centrifugation.

6. Assemble a HiBind® MicroElute column in a 2 ml collection tube (provided). Transfer the entire solution from Step 5 into the column, including any precipitate that may have formed. Close the lid 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 new collection tube (supplied). Add 500µl of Buffer HB in the column. Close the lid and centrifuge at 8000 x g for 1 minute. Discard the flow-through and re-use collection tube.

8. Place the column into **the same 2 ml tube** (supplied) and wash by pipetting 650 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: *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.*

9. Using a **new collection tube**, wash the column with a second 650 µl of DNA Wash Buffer ***diluted with ethanol*** and centrifuge as above. Discard flow-through and re-use the collection tube.

10. Using the same 2ml collection tube, centrifuge empty column at maximum speed (≥20,000 x g) for 3 min to dry the HiBind® membrane. ***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 10-50µl of preheated (70ºC) Elution Buffer or water onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.

12. To elute DNA from the column, centrifuge at 20,000 x g for 1 min.

E.Z.N.A.® Protocol For 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 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 0.5 ml per sample) at 70ºC.

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 1.5 or 2.0ml centrifuge tube. (not provided).
   Note: Use 1-3 punched circles (3mm diameter) for each DNA isolation.

2. Add 200 µl Buffer TL to 1-3 of 3 mm punched filter paper circle. Follow by addition of 20 µl OB Protease solution. Incubate mixture at 60ºC for 45-60 minutes. Mix the samples several times during incubation by vortexing.

3. Briefly centrifuge the centrifuge tube to bring down any liquid drop from inside of the lid.

4. Add 220 µl Buffer BL, close the lid and mix throughly by vorexting 20s at maxi speed. 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 10 seconds few times during incubation.

6. Briefly centrifuge the centrifuge tube to bring down any liquid drop from inside of the lid.
   Note: For maximum yield, Collection any remaining liquid from paper, transfer all entire sample include paper into a Homogenizer Column (not supplied) and centrifuge at 20,000 x g for 2 minutes to collect all the lysates. Homogenizer column can be purchased separately from Omega Bio-tek (Product No. HCR-001 an HCR-003).

7. **Add 220 µl absolute ethanol and mix thoroughly by vortexing 20s at maxi speed.** Centrifuge briefly to bring down any liquid from inside of lid.

8. Assemble a HiBind® MicroElute column in a 2 ml collection tube (provided). Transfer the entire lysate from Step 7 into the HiBind DNA MicroElute column including any precipitate. Close the lid and centrifuge at 8,000 x g for 2 min to bind DNA. Discard the collection tube and flow-through liquid.

9. Place the column into a new collection tube (supplied). Add 500µl of Buffer HB in the column. Close the lid and centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.

10. Place the column into a **new 2 ml tube** (supplied) and wash by pipetting 650 µl of DNA Wash Buffer ***diluted with ethanol***. Centrifuge at 8,000 x g for 1 min. Discard the flow-through 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.*

11. Using a **same collection tube from step 10**, wash the column with a second 650 µl of DNA Wash Buffer ***diluted with ethanol*** and centrifuge as above. Discard flow-through and re-use the collection tube.

12. Using the same 2ml collection tube, centrifuge the empty column at maximum speed (15,000 x g) for 3 min to dry the HiBind® membrane. ***This step is crucial for ensuring optimal elution in the following step.***

13. Place the column into a sterile 1.5 ml microfuge tube and **add 10-50µl of preheated (70ºC) Elution Buffer or water 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 20,000 x g for 1 min.

    Note: Incubation at 70ºC 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 10-50µl of preheated Elution Buffer or water.

E.Z.N.A.® Protocol for Isolation of Genomic DNA from Swab

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

1. Place the swab in a 2 ml microcentrifuge tube.

2. **Add 600µl Buffer TL and 20µl Protease solution into the tube**. Mix throughly by vortexing for 30 seconds at maxi speed.

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 the tube to spin down any liquid drop from inside of the lid.

5. **Add 620µl Buffer BL, close the lid, mix throughly by vortexing**. If Linear Acrylamide is needed, add 4µl of dissolved Linear Acrylamide to 660µl Buffer BL. See page 3 for detailed 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 µl absolute ethanol and mix thoroughly by vortexing for 20s at maxi speed.** Centrifuge briefly to bring down any liquid from inside of lid.

9. Assemble a HiBind® MicroElute column in a 2 ml collection tube (provided). Transfer the 700µl of lysate from Step 8 into the column including any precipitate that may have formed. Close the lid and centrifuge at 8,000 x g for 1 min to bind DNA. Discard flow-through liquid and re-use the collection tube.

10. Place the HiBind® MicroElute column into the same collection tube from step 9 and repeat step 9 until all of the remaining lysate from step 8 has passed 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 x g for 2 minutes to collect remaining lysates. Homogenizer column can be purchased separately from Omega Bio-tek (Product No. HCR-001 an HCR-003).

11. Place the column into a new collection tube (supplied). Add 500µl of Buffer HB in the column. Close the lid and centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.

12. Place the column into a **new 2 ml tube** (supplied) and wash by pipetting 650 µl of DNA Wash Buffer ***diluted with ethanol***. Centrifuge at 8,000 x g 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.*

13. Using the **same collection tube from step 12**, wash the column with a second 650 µl of DNA Wash Buffer ***diluted with ethanol*** and centrifuge as above. Discard flow-through and re-use the collection tube.

14. Using the same 2ml collection tube, centrifuge empty column at maximum speed (20,000 x g) for 3 min to dry the HiBind® membrane. ***This step is crucial for ensuring optimal elution in the following step.***

15. Place the column into a sterile 1.5 ml microfuge tube and add 10-50µl of preheated (70ºC) Elution Buffer or water onto the center of the membrane. Allow tubes to sit for 3 min at room temperature.

16. To elute DNA from the column, centrifuge at 20,000 x g for 1 min.

Protocol for 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 300µl of Buffer TL into the tube, mix throughly by vortexing**. If process semen stints, add 20µl of DTT for each sample.

2. **Add 20µl of OB Protease solution to each sample**. Incubate sample at 60ºC for 45-60 minutes or over night 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 µl Buffer BL, close the lid and mix throughly by vortexing for 20 seconds**. If only one punch card is processed, add 4µl of Linear Acrylamide to the sample. See page 3 for detailed 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 x g 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. Assemble a HiBind® MicroElute column in a 2 ml collection tube (provided). Transfer the 600µl of sample from Step 6 into the column including any precipitate that may have formed. Close the lid and centrifuge at 8,000 x g for 1 min to bind DNA. Discard flow-through liquid and re-use the collection tube.

8. Place the HiBind® MicroElute column into the same collection tube from step 7 and transfer the remaining lysate from step 6 into the column. Centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.

9. Place the column into a new collection tube (supplied). **Add 500µl of Buffer HB in the column**. Close the lid and centrifuge at 8000 x g for 1 minute. Discard the flow-through and collection tube.

10. Place the column into a **new 2 ml tube** (supplied) and wash by pipetting 650 µl of DNA Wash Buffer ***diluted with ethanol***. Centrifuge at 8,000 x g for 1 min. Discard the flow-through and re-use the collection tube.

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

11. Using the **same collection tube from step 10**, wash the column with a second 650 µl of DNA Wash Buffer ***diluted with ethanol*** and centrifuge as above. Discard flow-through and re-use the collection tube.

12. Using the same 2ml collection tube, centrifuge empty column at maximum speed (20,000 x g) for 3 min to dry the HiBind® membrane. ***This step is crucial for ensuring optimal elution in the following step.***

13. Place the column into a sterile 1.5 ml microfuge tube and add 10-50µl of preheated (70ºC) Elution Buffer or water 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 20,000 x g for 1 min.

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 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.**
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol
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.
	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	Increase incubation time with Buffer TL and protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein.	**After applying to column, wash with Buffer HB twice then with DNA Wash Buffer.**
No DNA eluted	Improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading 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	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 .	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.