E.Z.N.A.® Tissue DNA Kit

Revision No: 3.1

omega BIO-TEK

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D3396-00	D3396-01	D3396-02	D3396-03
Purifications	5	50	200	600
HiBind® DNA Mini Columns	5	50	200	600
2 mL Collection Tubes	10	100	400	1200
BL Buffer	5 mL	20 mL	60 mL	2 x 100 mL
TL Buffer	5 mL	20 mL	60 mL	135 mL
HBC Buffer	5 mL	25 mL	80 mL	250 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL	200 mL
Elution Buffer	15 mL	30 mL	2 x 60 mL	300 mL
Proteinase K Solution	150 µL	1.5 mL	6 mL	18 mL

Supplied by user:

- Tabletop microcentrifuge capable of 13,000*g*
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 100% isopropanol
- PBS for cultured cells protocol
- Optional: RNase stock solution (100 mg/mL)

Before starting:

- Set water baths, heat blocks, or incubators to 55°C and 70°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions on the bottles.
- Chill PBS to 4°C for cultured cells protocol.
- Heat Elution Buffer to 70°C.

DNA Extraction and Purification from Tissue

LYSE

This method is suitable for the isolation of DNA from up to 30 mg tissue. Yields vary depending on source. The protocol can be scaled up to accomodate larger samples, but additional TL Buffer (Cat.#PD061) and BL Buffer (Cat.#PD062) will need to be purchased separately.

1. Mince up to 30 mg tissue and transfer to a 1.5 mL microcentrifuge tube.
2. Add 200 µL TL Buffer. For samples >30 mg, increase the volume of TL Buffer; for a 40-60 mg sample use 400 µL TL Buffer.
3. Add 25 µL Proteinase K Solution. Vortex to mix thoroughly.
4. Incubate at 55°C in a shaking water bath. The average time is less than 3 hours. Lysis can proceed overnight. If a shaking water bath is not available, vortex the sample every 20-30 minutes.

   OPTIONAL: Certain tissues such as liver tissue 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.

   1. Add 4 µL RNase A (100 mg/mL) per 30 mg tissue.
   2. Let sit at room temperature for 2 minutes.
   3. Proceed to Step 5 below.

5. Centrifuge at maximum speed (≥10,000*g*) for 5 minutes.
6. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.

BIND

7. Add 220 µL BL Buffer. Adjust the volume of BL Buffer based on the amount of starting material and volume of TL Buffer used (only for Tissue protocol). Vortex to mix thoroughly. A wispy precipitate may form upon the addition of BL Buffer. This is does not interfere with DNA recovery.
8. Incubate at 70°C for 10 minutes.

BIND

9. Add 220 µL 100% ethanol. Adjust the ethanol volume accordingly if increased volume of TL Buffer and BL Buffer are used (only for Tissue protocol). Vortex to mix thoroughly.
10. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
11. Transfer the entire sample from Step 9 to the HiBind® DNA Mini Column including any precipitates that may have formed.

WASH

12. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
13. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions).
14. Centrifuge at maximum speed for 30 seconds. Discard the filtrate and collection tube.
15. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.
16. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.
17. Repeat Step16 for a second DNA Wash Buffer wash step.
18. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.

ELUTE

19. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).
20. Add 100-200 µL Elution Buffer heated to 70°C. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.
21. Repeat Step 20 for a second elution step.
22. Store eluted DNA at -20°C.

DNA Extraction and Purification from Cultured Cells

LYSE

1. Prepare the cell suspension using one of the following methods for purification of genomic DNA from up to 5 x 10^6 cells.

   A) Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   B) For cells grown in suspension, pellet 5 x 10^6 by spinning at 1,200*g* in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 200 µL PBS. Proceed to Step 2.

   C) For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 200 µL PBS. Proceed to Step 2.

2. Add 25 µL Proteinase K Solution. Vortex to mix thoroughly.

   Optional: Cultured cells 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.

   1. Add 4 µL RNase A (100 mg/mL) per 5 x 10^6 cells.
   2. Let sit at room temperature for 2 minutes.
   3. Proceed to Step 3 below.

3. Proceed to Step 7 of the DNA EXTRACTION AND PURIFICATION FROM TISSUE protocol on the reverse page.