E.Z.N.A.® Blood DNA Mini Kit

Revision No: 1.2

Quick Guide

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

Product	D3392-00	D3392-01	D3392-02	D3392-03
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	3 x 110 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	60 mL	250 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*
- Water bath, heat block, or incubator capable of 65°C
- Vortexer
- Nuclease-free 2 mL microcentrifuge tubes
- 100% ethanol
- 100% isopropanol
- PBS if using the Buccal Swab or Dried Blood Protocol
- Optional: 10 mM Tris-HCl or PBS
- Optional: RNase stock solution (50 mg/mL)

Before starting:

- Set water bath, heat block, or incubator to 65°C.
- Prepare DNA Wash Buffer and HBC Buffer according to the directions on the bottles.
- Heat Elution Buffer to 65°C.

DNA Extraction and Purification from Blood and Body Fluids

The procedure below has been optimized for the use with fresh or frozen blood samples up to 250 µL in volume. Anti-coagulated blood, saliva, serum, buffy coat, or other body fluids can also be used. In addition, ≤10^7 leukocytes or cultured cells may be used.

LYSE

1. Transfer the sample into a nuclease-free 2 mL microcentrifuge tube (not provided) and bring the volume up to 250 µL with 10mM Tris-HCl, PBS, or Elution Buffer.
2. Add 25 µL Proteinase K Solution and 250 µL BL Buffer. Vortex at maximum speed for 15 seconds. **Optional:** If RNA-free genomic DNA is required, add 5 µL RNase A (50 mg/mL).
3. Incubate at 65°C for 10 minutes. Vortex briefly once during incubation.

BIND

4. Add 260 µL 100% ethanol. Vortex at maximum speed for 20 seconds.
5. Centrifuge briefly to collect any drops from the inside of the lid.
6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
7. Transfer the entire sample to the column.
8. Centrifuge at ≥10,000*g* for 1 minute.
9. Discard the filtrate and the 2 mL Collection Tube.

WASH

10. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.
11. Add 500 µL HBC Buffer diluted with 100% isopropanol (see the bottle for instructions). Centrifuge at ≥10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
12. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at ≥10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
13. Repeat Step12 for a second DNA Wash Buffer Step.
14. 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

15. Transfer the HiBind® DNA Mini Column into a nuclease-free 2 mL microcentrifuge tube.
16. Add 100-200 µL Elution Buffer heated to 65°C. Let sit at room temperature for 5 minutes. Centrifuge at maximum speed for 1 minute.
17. Repeat Step 16 for a second elution step.

    Note: The second elution step can either be repeated with fresh Elution Buffer (this may increase the yield, but decrease the concentration) or eluate from the first elution (this may increase yield while maintaining elution volume).
18. Store eluted DNA at -20°C.

DNA Extraction and Purification from Buccal Swabs

This protocol requires an increased volume of BL Buffer. Fewer preparations can be performed. Additional BL Buffer can be purchased separately (Cat#PD062).

LYSE

1. Place the buccal swab in a nuclease-free 2 mL microcentrifuge tube. Add 500 µL PBS. **Optional:** If RNA-free genomic DNA is required, add 5 µL RNase A (50 mg/mL).
2. Add 25 µL Proteinase K Solution and 500 µL BL Buffer. Vortex at maximum speed for 30 seconds. Incubate at 65°C for 10 minutes.
3. Discard the buccal swab.

BIND

4. Add 500 µL 100% ethanol. Vortex at maximum speed for 20 seconds. Centrifuge briefly to collect any drops from the inside of the lid.
5. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
6. Transfer 750 µL sample to the column. Centrifuge at 10,000*g* for 1 minute. Discard the filtrate and reuse the collection tube.
7. Repeat Step 6 until all the sample has been transferred to the column.
8. Proceed to Step 9 of the DNA EXTRACTION AND PURIFICATION FROM BLOOD AND BODY FLUIDS protocol on the reverse page.

DNA Extraction and Purification from Dried Blood

LYSE

1. Cut or punch-out the blood spot from the filter paper (up to 200 µL blood can be used per spot). Tear or cut the filter paper into small pieces and place them into a nuclease-free 2 mL microcentrifuge tube.
2. Add 250 µL PBS. Incubate at 65°C for 1 hour. Vortex briefly every 20 minutes.
3. Add 25 µL Proteinase K Solution. Vortex at maximum speed for 15 seconds.
4. Incubate at 65°C for 30 minutes. Vortex briefly several times during incubation. Centrifuge at ≥13,000*g* for 5 minutes.
5. Transfer the supernatant to a nuclease-free 2 mL microcentrifuge tube.

BIND

6. Add 1 volume BL Buffer and 1 volume 100% ethanol. Vortex to mix thoroughly. Centrifuge briefly to collect any drops from the inside of the lid.
7. Proceed to Step 6 of the DNA EXTRACTION AND PURIFICATION FROM BLOOD AND BODY FLUIDS protocol on the reverse page.