# Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

Revision No: 2.2

## Quick Guide

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

| Product | M6399-00 | M6399-01 | M6399-02 |
|---|---|---|---|
| Purifications | 1 x 96 | 4 x 96 | 24 x 96 |
| AL Buffer | 35 mL | 125 mL | 725 mL |
| TL Buffer | 30 mL | 120 mL | 600 mL |
| HDQ Binding Buffer | 10 mL | 40 mL | 200 mL |
| VHB Buffer | 66 mL | 230 mL | 3 x 440 mL |
| SPM Buffer | 30 mL | 150 mL | 3 x 150 mL |
| Elution Buffer | 60 mL | 250 mL | 1000 mL |
| Proteinase K Solution | 2.2 mL | 9 mL | 50 mL |
| Mag-Bind® Particles HDQ | 2.2 mL | 9 mL | 50 mL |

**Important:**

If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

Kits include enough reagents for the specified number of preparations plus an additional 10% overage to ensure there is sufficient volume. Please be aware that the actual number of preparations may be lower due to pre-aliquoting of reagents, processing partial plates, and automation platform used etc. Additional reagents are available for purchase separately. Please visit the product page at www.omegabiotek.com or contact your Omega Bio-tek representative for more details and ordering information.

**Supplied by user:**

- Magnetic separation device (Recommend Alpaqua#A000380)
- Heat block/incubator/water bath capable of 70°C
- Vortexer
- 96-well Microplate (500 µL) or elution plate
- 96-well deep-well plates (Recommend Nunc #278752)
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- If using the Tissue Protocol:
  - Shaking water bath capable of 55°C
  - Centrifuge with swing-bucket rotor and plate adapter capable of 4,000*g*
- Recommended: 1M Dithiothreitol (DTT)
- Optional: RNase (10 mg/mL), PBS, liquid nitrogen, mortar and pestle

**Before starting:**

- Set water bath, heat block, or incubator to 55°C and/or 70°C.
- Prepare HDQ Binding Buffer, VHB Buffer, and SPM Buffer according to the directions on the bottles.
- Vortex Mag-Bind® Particles HDQ to completely resuspend.

## DNA Extraction and Purification from 250 µL Fresh or Frozen Blood

LYSE

1. Prepare AL Buffer/Proteinase K Solution mastermix by mixing 30.6 mL AL Buffer with 2.1 mL Proteinase K Solution. This will make enough mastermix for one 96-well plate and can be scaled up or down based on sample number. Only prepare as much mastermix that will be used within 4 hours of preparation.
2. Add 250 µL blood sample to a 2 mL 96-well deep-well plate (not provided). If the volume of blood is less than 250 µL, bring the volume up to 250 µL with PBS (not provided) or Elution Buffer (provided with this kit).
3. Add 310 µL AL Buffer/Proteinase K Solution mastermix to each sample. Vortex or pipet up and down 20 times to mix. Proper mixing is crucial for good yield. For automated protocols tip mixing yields best results and is recommended.
4. Incubate at 70°C for 10 minutes.

   **Optional:** Add 5 µL RNase A. Vortex to mix. Let sit at room temperature for 2 minutes.

BIND

5. Add 400 µL HDQ Binding Buffer diluted with 100% isopropanol (see the bottle for instructions) and 20 µL Mag-Bind® Particles HDQ. Vortex for 10 minutes to mix. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed for each run. If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.
6. Place the plate on a magnetic separation device. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.
7. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ. Remove the plate from the magnetic separation device.

WASH

8. Add 600 µL VHB Buffer diluted with 100% ethanol (see the bottle for instructions). Vortex for 15 seconds to mix. Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.
9. Place the plate on the magnetic separation device. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely

WASH

cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ. Remove the plate from the magnetic separation device.
11. Repeat Steps 8-10 for a second VHB Buffer step.
12. Add 600 µL SPM Buffer diluted with 100% ethanol (see the bottle for instructions). Vortex for 15 seconds to mix.
13. Place the plate on the magnetic separation device. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.
14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.
15. Select one of the following ethanol removal steps:

    A. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water (not provided), leave on magnet for 20-30 seconds, and then aspirate. Do not leave nuclease-free water on Mag-Bind® Particles HDQ for more than 60 seconds. Remove the plate from the magnetic separation device. Continue to Step 16.

    **OR**

    B. Leave the plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipettor. Dry the Mag-Bind® Particles HDQ for an additional 10 minutes. Remove the plate from the magnetic separation device. Continue to Step 16.

ELUTE

16. Add 50-200 µL Elution Buffer or nuclease-free water. Vortex for 5 minutes to mix. Heat Elution Buffer to 70°C to improve yield. If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.
17. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles HDQ. Let sit at room temperature until the Mag-Bind® Particles HDQ are completely cleared from solution.
18. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

## DNA Extraction and Purification from up to 10 mg Tissue

LYSE

1. Mince up to 10 mg tissue and transfer to a 2 mL 96-well deep-well plate (not provided). Cutting the tissue into small pieces can speed up lysis.
2. Add 250 µL TL Buffer. Adding DTT to TL Buffer to a final concentration of 40 µM (40 µL 1M DTT per 1 mL TL Buffer) can help with tissue lysis and is recommended for lysis of hair or other tough-to-lyse tissues.
3. Add 20 µL Proteinase K Solution. Vortex to mix.
4. Incubate at 55°C in a shaking water bath. If a shaking water bath is not available, vortex the sample every 20-30 minutes. Lysis time depends on amount and type of tissue, but is usually under 3 hours. The lysis can proceed overnight.

   **Optional:** Add 5 µL RNase A. Vortex to mix. Let sit at room temperature for 2 minutes.

BIND

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair. Carefully transfer 200 µL supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.
6. Add 230 µL AL Buffer. Vortex for 10 minutes or pipet up and down 20 times to mix. Proper mixing is crucial for good yield. For automated protocols, tip mixing yields best results and is recommended. If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.
7. Add 320 µL HDQ Binding Buffer diluted with 100% isopropanol (see the bottle for instructions) and 20 µL Mag-Bind® Particles HDQ. Vortex for 10 minutes to mix. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Mix only what is needed for each run. If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.
8. Proceed to Step 6 of the DNA EXTRACTION AND PURIFICATION FROM 250 µL FRESH OR FROZEN BLOOD protocol on the reverse page.