omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

M6399-00	1 x 96 preps
M6399-01	4 x 96 preps

Manual Date: May 2021
Revision Number: v8.1

For Research Use Only

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Mag-Bind® Blood & Tissue DNA HDQ 96 Kit

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Introduction

Mag-Bind® Blood & Tissue DNA HDQ 96 Kit offers a versatile method for the isolation of high-quality DNA from a wide variety of samples including fresh or frozen animal cultured cells and tissues, up to 250 µL whole blood, buccal swabs, up to 500 µL saliva, mouse tail snips, and dried blood spots. Mag-Bind® Particles HDQ provide a quick magnetic response time reducing overall processing time. This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with the time-proven efficiency of Omega Bio-tek's buffer chemistries to provide a fast and convenient method to isolate DNA from a variety of samples. The purification procedure provides high-quality DNA that is suitable for direct use in most downstream applications, such as amplification, next generation sequencing and enzymatic reactions.

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

Overview

If using the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Samples are lysed in buffer systems that are tailored specifically for each type of starting material. After lysis, samples are mixed with HDQ Binding Buffer and Mag-Bind® Particles HDQ to bind DNA to the magnetic beads. The paramagnetic particles are separated from the lysates by using a magnetic separation device. After a few rapid wash steps to remove trace contaminants, DNA is eluted in Elution Buffer.

New in this Edition:

March 2020:

- Protocol modifications were made to the Saliva Protocol to accomodate 500 µL sample volume.

June 2019:

- SPM Wash Buffer has been renamed SPM Buffer. This is a name change only. The formulation has not changed.

August 2018:

- An optional method to dry Mag-Bind® Particles HDQ has been added to Blood, Tissue, Cultured Cells, Saliva, and Tail Snips extraction protocols.
- Addition of recommended mastermix of AL Buffer and Proteinase K Solution has been added to Blood, Cultured Cells, Saliva, and Buccal Swab extraction protocols.

Kit Contents

Product	M6399-00	M6399-01
Preps	1 x 96	4 x 96
Mag-Bind® Particles HDQ	2.2 mL	9 mL
AL Buffer	35 mL	125 mL
TL Buffer	30 mL	120 mL
HDQ Binding Buffer	10 mL	40 mL
VHB Buffer	66 mL	230 mL
SPM Buffer	30 mL	150 mL
Proteinase K Solution	2.2 mL	9 mL
Elution Buffer	60 mL	250 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles HDQ should be stored at 2-8°C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. Store all other components at room temperature.

Magnetic Separation Devices and Plasticware

While many brands of magnetic separation devices are compatible with the Mag-Bind® Blood & Tissue DNA HDQ 96 Kit, we recommend using Alpaqua's Magnum™ EX Universal Magnet Plate (Part# A000380) in conjunction with Nunc 2 mL DeepWell™ plates (Part# 278752). This combination provides quick magnetization times, only 1 minute for complete magnetization during wash steps and 5 minutes for lysate clearance steps.

Regardless of the magnetic separation device selected, ensure the device is compatible with the plasticware necessary for this kit.

Preparing Reagents

1. Dilute SPM Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6399-00	70 mL
M6399-01	350 mL

2. Prepare VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6399-00	84 mL
M6399-01	290 mL

3. Prepare HDQ Binding Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
M6399-00	40 mL
M6399-01	160 mL

4. Shake or vortex the Mag-Bind® Particles HDQ to fully resuspend the particles before use. The particles must be fully suspended during use to assure proper binding.

Mag-Bind® Blood & Tissue DNA 96 Kit - 250µL Blood Protocol

The procedure below has been optimized for use with 250 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

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

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- Heat block, incubator, or water bath capable of 70°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plate (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: PBS

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set heat block, incubator, or water bath to 70°C.

1. Prepare a mastermix of AL Buffer and Proteinase K Solution only for samples to be extracted according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
AL Buffer	290 µL	30.6 mL*
Proteinase K Solution	20 µL	2.1 mL*

* 10% excess volume has been calculated for a 96-well plate.

Important: Only prepare as much AL Buffer/Proteinase K Solution mastermix that will be used within 4 hours of preparation.

2. Add blood samples to a 2 mL 96-well deep-well plate (not provided). Bring the volume up to 250 µL with PBS (not provided) or Elution Buffer if volume of blood is less than 250 µL.

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.

   Note: 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 to each sample. Vortex to mix. Let sit at room temperature for 2 minutes.

5. Add 400 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

   Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

   Note: 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 to magnetize the Mag-Bind® Particles HDQ. 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.

8. Remove the plate from the magnetic separation device.

9. Add 600 µL VHB Buffer to each sample.

   Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

10. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

11. 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.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

13. Remove the plate from the magnetic separation device.

14. Repeat Steps 9-13 for a second VHB Buffer wash step.

15. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

16. Vortex for 15 seconds to mix.

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. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

19. 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. Continue to Step 20.

    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. Continue to Step 20.

20. Remove the plate from the magnetic separation device.

21. Add 50-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

22. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

23. 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.

24. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Tissue Protocol

This method allows genomic DNA isolation from up to 10 mg tissue. Yields will vary depending on the source.

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

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: Liquid nitrogen and mortar and pestle
- Recommended: 1M Dithiothreitol (DTT)

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set water bath to 55°C.
- Optional: Set water bath, incubator, or heat block 70°C.
- Recommended: Add 40 µL 1M DTT per 1 mL TL Buffer before use.

OPTIONAL: Although mechanical homogenization of tissue is not 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 96-well deep-well plate (not provided). Add 250 µL TL Buffer and proceed to Step 3 on the next page.

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

1. Mince up to 10 mg tissue and transfer to a 96-well deep-well plate (not provided).

   Note: Cutting the tissue into small pieces can speed up lysis.

2. Add 250 µL TL Buffer to each sample.

   Optional: For lysis of hair or other tough-to-lyse tissues, a mastermix of TL Buffer and DTT is recommended.
   - Dilute DTT to a final concentration of 40 mM in TL Buffer.
   - Add 40 µL 1M DTT per 1 mL TL Buffer before use.
   - Only prepare as much TL Buffer/DTT mastermix that will be used immediately.

3. Add 20 µL Proteinase K Solution to each sample. Vortex to mix.

4. Incubate at 55°C in a shaking water bath.

   Note: 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 to each sample. Vortex to mix. Let sit at room temperature for 2 minutes.

5. Centrifuge at maximum speed (≥4,000 x *g*) for 5 minutes to pellet undigested tissue debris.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

7. Add 230 µL AL Buffer to each sample. Vortex for 10 minutes to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols tip mixing yields best results and is recommended.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

8. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

   Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

9. Place the plate on a 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.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

11. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

12. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

13. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

14. 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.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

16. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

17. Repeat Steps 12-16 for a second VHB Buffer wash step.

18. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

19. Vortex for 15 seconds to mix.

20. 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.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

22. 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. Continue to Step 23.

    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. Continue to Step 23.

23. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

25. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

26. 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.

27. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Cultured Cells Protocol

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

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

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- Shaking water bath capable of 55°C
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Cold PBS (4°C)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: Trypsin and cell scraper

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set shaking water bath to 55°C.
- Optional: Set water bath, incubator, or heat block 70°C.

Mag-Bind® Blood & Tissue DNA HDQ 96 Kit
Cultured Cells Protocol

1. Prepare the cell suspension.

   1a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation. Wash the cells with cold PBS (4°C) and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1b. For cells grown in suspension, pellet 5 x 10^6 cells at 1,200 x *g* in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   1c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Wash cells twice in cold PBS (4°C) and resuspend the cells with 180 µL cold PBS. Proceed with Step 2 of this protocol.

2. Prepare a mastermix of AL Buffer and Proteinase K Solution only for samples to be extracted according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
AL Buffer	230 µL	24.3 mL*
Proteinase K Solution	20 µL	2.1 mL*

* 10% excess volume has been calculated for 96-well plate.

   Important: Only prepare as much AL Buffer/Proteinase K Solution mastermix that will be used within 4 hours of preparation.

3. Add 250 µL AL Buffer/Proteinase K Solution mastermix to each sample. Vortex for 10 minutes to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols tip mixing yields best results and is recommended.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the samples every 2-3 minutes.

5. Transfer the samples to a 96-well deep-well plate (not provided).

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

6. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

   Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

7. Place the plate on a 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.

8. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

9. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

10. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

11. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

12. 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.

13. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

14. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

15. Repeat Steps 10-14 for a second VHB Buffer wash step.

16. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

17. Vortex for 15 seconds to mix.

18. 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.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

20. 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. Continue to Step 21.

    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. Continue to Step 21.

21. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

22. Add 50-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

23. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

24. 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.

25. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Saliva Protocol

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

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Shaking water bath capable of 55°C
- Optional: RNase A (10mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set shaking water bath to 55°C.
- Optional: Set water bath, incubator, or heat block 70°C.

1. Centrifuge the saliva tube at 2,000 x *g* for 5 minutes.

2. Transfer 500 µL stabilized saliva samples (e.g. DNA Genotek Oragene®, Mawi iSWAB™, Biomatrica® DNAgard® Saliva) to a 96-well deep-well plate (not provided).

3. Prepare a mastermix of AL Buffer and Proteinase K Solution only for samples to be extracted according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
AL Buffer	200 µL	21.12 mL*
Proteinase K Solution	20 µL	2.1 mL*

* 10% excess volume has been calculated for 96-well plate.

Important: Only prepare as much AL Buffer/Proteinase K Solution mastermix that will be used within 4 hours of preparation.

4. Add 220 µL AL Buffer/Proteinase K Solution to each sample. Vortex for 10 minutes to mix. Proper mixing is crucial for good yield.

Note: For automated protocols tip mixing yields best results and is recommended.

Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

5. Incubate at 55°C in a shaking water bath for 10 minutes.

Note: If a shaking water bath is not available, vortex the plate every 2-3 minutes. If DNA Genotek Oragene® tube was used and incubation step was already performed, skip to Step 6.

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

6. Add 400 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

7. Place the plate on a 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.

8. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

9. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

10. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

11. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

12. 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.

13. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

14. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

15. Repeat Steps 10-14 for a second VHB Buffer wash step.

16. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

17. Vortex for 15 seconds to mix.

18. 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.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

20. 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. Continue to Step 21.

    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. Continue to Step 21.

21. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

22. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

23. 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.

24. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Buccal Swabs Protocol

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

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Nuclease-free water
- Optional: Heat block, incubator, or water bath capable of 70°C

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set shaking water bath to 55°C.
- Optional: Set water bath, incubator, or heat block 70°C.

1. Cut off the buccal brush or swab head and place each swab into a well of a 96-well deep-well plate (not provided).

2. Prepare a mastermix of AL Buffer, Proteinase K Solution, and Elution Buffer only for samples to be extracted according to the table below:

Component	Amount per Prep	Total Amount per 96-well Plate
AL Buffer	290 µL	30.6 mL*
Proteinase K Solution	20 µL	2.1 mL*
Elution Buffer	250 µL	26.4 mL

* 10% excess volume has been calculated for a 96-well plate.

Important: Only prepare as much AL Buffer/Proteinase K Solution/Elution Buffer mastermix that will be used within 4 hours of preparation.

3. Add 560 µL AL Buffer/Proteinase K Solution/Elution Buffer mastermix to each sample. Vortex or pipet up and down 20 times to mix.

   Note: For automated protocols tip mixing yields best results and is recommended.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the plate every 2-3 minutes.

5. Centrifuge at 3,000 x *g* for 2 minutes.

6. Transfer 500 µL lysate into a new 96-well deep-well plate. Do not transfer the swabs to the new plate.

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

7. Add 350 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

   Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

8. Place the plate on a 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.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

10. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

11. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

12. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

13. 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.

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

15. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

16. Repeat Steps 11-15 for a second VHB Buffer wash step.

17. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

18. Vortex for 15 seconds to mix.

19. 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.

20. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

21. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles HDQ. Remove any residual liquid from the wells.

    Note: All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait one minute and remove any residual liquid from the well.

22. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

23. Add 100-200 µL Elution Buffer or nuclease-free water (not provided) to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

24. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

25. 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.

26. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Mag-Bind® Blood & Tissue DNA 96 Kit - Mouse Tail Snips Protocol

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

Materials and Equipment to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device (Recommend Alpaqua Magnum™ EX, Part#A000380)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x *g*
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Microplate (500 µL) (Cat# EZ9604) or desired elution plate
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film (Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Recommended: 1M Dithiothreitol (DTT)

Before Starting:

- Prepare SPM Buffer, VHB Buffer, and HDQ Binding Buffer according to the "Preparing Reagents" section on Page 4.
- Set water bath to 55°C.
- Recommended: Add 40 µL 1M DTT per 1 mL TL Buffer.

1. Snip a 2-5 mm piece of mouse tail, cut into several pieces, and transfer the pieces to a 96-well deep-well plate (not provided).

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks since lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 250 µL TL Buffer to each sample.

   Optional: For lysis of hair or other tough-to-lyse tissues, a mastermix of TL Buffer and DTT is recommended.
   - Dilute DTT to a final concentration of 40 mM in TL Buffer.
   - Add 40 µL 1M DTT per 1 mL TL Buffer before use.
   - Only prepare as much TL Buffer/DTT mastermix that will be used immediately.

3. Add 20 µL Proteinase K Solution to each sample. Vortex to mix.

4. Incubate at 55°C in a shaking water bath for 1-4 hours or until lysis is complete.

   Note: If a shaking water bath is not available, vortex the samples vigorously every 20-30 minutes. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail snip and age of animal, e.g. a 5 mm tail piece from a 2 week old mouse typically will lyse in 2 hours. For older animals, an overnight incubation may improve yields. Note that bone and hair will not lyse. Adding DTT to TL Buffer at a final concentration of 40 mM can help with tissue lysis.

5. Centrifuge at maximum speed (≥4,000 x *g*) for 5 minutes to pellet undigested tissue debris.

6. Carefully transfer 200 µL supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

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

7. Add 230 µL AL Buffer to each sample. Vortex for 10 minutes to mix. Proper mixing is crucial for good yield.

   Note: For automated protocols tip mixing yields best results and is recommended.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

8. Add 320 µL HDQ Binding Buffer and 20 µL Mag-Bind® Particles HDQ to each sample. Vortex for 10 minutes to mix.

   Note: HDQ Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 4 for instructions. HDQ Binding Buffer and Mag-Bind® Particles HDQ can be prepared as a mastermix. Prepare only what is needed for each run.

   Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes.

9. Place the plate on a 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.

10. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

11. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

12. Add 600 µL VHB Buffer to each sample.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

13. Vortex for 15 seconds to mix.

    Note: Complete resuspension of the Mag-Bind® Particles HDQ is critical for obtaining good purity.

14. 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.

15. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

16. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

17. Repeat Steps 12-16 for a second VHB Buffer wash step.

18. Add 600 µL SPM Buffer to each sample.

    Note: SPM Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

19. Vortex for 15 seconds to mix.

20. 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.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles HDQ.

22. 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. Continue to Step 23.

    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. Continue to Step 23.

23. Remove the plate containing the Mag-Bind® Particles HDQ from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the Mag-Bind® Particles HDQ.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

25. Vortex for 5 minutes to mix.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

26. 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.

27. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **(1-800-832-8896)**.

Problem	Cause	Solution
Low DNA yield	Incomplete resuspension of Mag-Bind® Particles HDQ	Resuspend the Mag-Bind® Particles HDQ by vortexing vigorously before use.
	Frozen blood samples not mixed properly after thawing	Thaw the frozen blood at room temperature and mix the blood by gently inverting the tube.
	Inefficient cell lysis due to inefficient mixing of AL Buffer/Proteinase K Solution mastermix and sample	Make sure the sample is thoroughly mixed with AL Buffer/Proteinase K Solution mastermix within 4 hours of mastermix preparation.
	Loss of Mag-Bind® Particles HDQ during operation	Avoid disturbing the Mag-Bind® Particles HDQ during aspiration.
	DNA remains bound to Mag-Bind® Particles HDQ	Increase elution volume and incubation time to 15 minutes; pipet up and down 50 to 100 times.
	DNA washed off	Dilute SPM Buffer by adding appropriate volume of ethanol prior to use (see Page 4 for instructions).
	Ethanol is not added to VHB buffer	Make sure to add ethanol to the VHB Buffer (see Page 4 for instructions).
Mag-Bind® Particles HDQ do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Gel-like material in the eluted DNA	Blood is too old	Remove the gel-like material by centrifugation; recommend using fresh blood.
		Use 8 mM NaOH as elution buffer.
Problems in downstream applications	Salt carryover	SPM Buffer must be at room temperature.
	Ethanol carryover	Dry the Mag-Bind® Particles HDQ before elution.

Notices & Disclaimers

For European Union Use.

AL Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or *in vitro* diagnostics.

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