omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

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

M6399-01PF96 4 x 96 preps

Manual Date: July 2021
Manual Revision: v1.2

For Research Use Only

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Introduction

Mag-Bind® Blood & Tissue DNA HDQ Prefilled 96 Kit includes prefilled plates in 96-well standard automation format and is configured to work seamlessly on magnetic processors such as KingFisher™ Flex, BioSprint®, and MagMAX® 96. Mag-Bind® Blood & Tissue DNA HDQ Prefilled 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, and up to 250 µL saliva. This kit enhances ease of use, convenience, and extraction accuracy and reduces hands-on time by skipping reagent preparation and buffer dispensing steps. The quick magnetic response of the Mag-Bind® Particles PF-HDQ along with no reagent set-up lessens the overall processing time. This system combines the reversible nucleic acid binding properties of Mag-Bind® Particles PF-HDQ 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 PCR amplification, next generation sequencing and enzymatic reactions.

If using Mag-Bind® Blood & Tissue DNA HDQ Prefilled 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. The lysates are then transferred to the iHDQ Binding Buffer plate and plates are loaded onto the magnetic processor for further processing. Briefly, the lysates are mixed with the Binding Buffer and Mag-Bind® Particles to bind DNA to the magnetic beads. The paramagnetic particles are separated from the lysates using magnetic particle separation technology of the magnetic processor being used. After a few rapid wash steps to remove trace contaminants, DNA is eluted in the Elution Buffer. The magnetic processor is programmed to perform the "bind-wash-elute" steps providing high-quality DNA suitable for a variety of downstream applications.

Important: Before starting extraction on a magnetic processor, please contact your Omega Bio-tek representative for scripts compatible with your specific instrument.

Kit Contents

Product	M6399-01PF96
Purifications	4 x 96 Preps
96-well Tip Comb	2 x 2 combs
96-well Deep-well Plate	4
AL Buffer	125 mL
TL Buffer	135 mL
iHDQ Binding Buffer Plate	4
eVHB Buffer Plate	8
eSPM Buffer Plate	4
Elution Buffer Plate	4
Mag-Bind® Particles PF-HDQ Plate	4
Proteinase K Solution	9 mL
User Manual	✓

Storage and Stability

All of the Mag-Bind® Blood & Tissue DNA HDQ Prefilled 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. 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 in an upright position. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Plasticware Handling and Preparation

1. Always check plates for the presence of precipitation before starting extraction. Dissolve precipitates by warming the plate at 37°C with gentle shaking.

2. Centrifuge all plates at 2,000*g* for 3 minutes to remove droplets from the seal.

3. Remove seal from plates and immediately load onto the instrument when prompted.

4. Load plates onto instrument according to Plate Layout table when prompted.

Pos.	Plate	Content	Volume per well
1	Mag-Bind® Particles PF-HDQ Plate	Mag-Bind® Particles PF-HDQ	100 µL
2	iHDQ Binding Buffer Plate	Lysate/iHDQ Binding Buffer	Lysate* + 400 µL
3	eVHB Buffer Plate	eVHB Buffer	600 µL
4	eVHB Buffer Plate	eVHB Buffer	600 µL
5	eSPM Buffer Plate	eSPM Buffer	600 µL
6	Elution Buffer Plate	Elution Buffer	100 µL
7	96-well Deep-well Plate	96-well tip comb nested in 96- well deep-well plate	n/a
8	n/a	n/a	n/a

*Volume will vary depending on extraction method.

Mag-Bind® Blood & Tissue HDQ 96 Prefilled Kit - 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: Before starting extraction on a magnetic processor, please contact your Omega Bio-tek representative for scripts compatible with your specific instrument.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing-bucket rotor capable of 2,000*g* and adaptor for 96-well plates
- Heat block, incubator, or water bath capable of 70°C
- Multichannel pipettes and reagent reservoirs
- 2 mL 96-well deep-well plate (Recommend ThermoFisher, Part #95040450 or Thomas Scientific, Part #20A00G709)
- 96-well microplate (Recommend ThermoFisher, Part #97002540)
- 1.5 mL microcentrifuge tubes
- Sealing film (Recommend Cat# AC1200)
- Optional: RNase A (10 mg/mL)
- Optional: PBS

Before Starting:

- Prepare each plate according to the "Plasticware Handling and Preparation" section on Page 4.
- Set heat block, incubator, or water bach to 70°C.

1. Prepare a mastermix of AL Buffer and Proteinase K Solution only for sample 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. Pipet up and down 20 times to mix thoroughly. Proper mixing is crucial for good yield.

4. Seal plate with sealing film (not provided.) Incubate at 70 °C for 10 minutes.

Optional: Add 5 µL RNase A and pipet up and down several times to mix thoroughly. Let sit at room temperature for 2 minutes.

5. Remove seal from iHDQ Binding Buffer plate and transfer lysate from Step 4 to plate.

   Note: Do not remove seals from remaining reagent plates until ready to load onto instrument in Step 7. Prepare plates according to the "Plasticware Handling and Preparation" section on Page 4.

6. Pipet up and down 5-10 times to mix thoroughly.

7. Start program on magnetic processor. Remove seals from remaining reagent plates and immediately load plates onto instrument according to Plate Layout table on Page 4 when prompted.

   Note: Prepare reagent plates according to the "Plasticware Handling and Preparation" section on Page 4.

8. After run has completed, remove the Elution Buffer Plate from instrument. Seal plate with sealing film or transfer the eluate to a new 1.5 mL tube (not provided) or 96-well microplate (not provided).

9. Store DNA at -20°C.

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

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

Important: Before starting extraction on a magnetic processor, please contact your Omega Bio-tek representative for scripts compatible with your specific instrument.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing-bucket rotor capable of 2,000*g* and adaptor for 96-well plates
- Vortexer with plate adaptor
- Shaking water bath capable of 55°C
- Multichannel pipettes and reagent reservoirs
- 2 mL 96-well deep-well plate (Recommend ThermoFisher, Part #95040450 or Thomas Scientific, Part #20A00G709)
- 96-well microplate (Recommend ThermoFisher, Part #97002540)
- 1.5 mL microcentrifuge tubes
- Sealing film (Recommend Cat# AC1200)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: Liquid nitrogen and mortar pestle
- Optional: RNase A (10 mg/mL)
- Recommended: 1M Dithiothreitol (DTT)

Before Starting:

- Prepare each plate according to the "Plasticware Handling and Preparation" section on Page 4.
- Set water bath to 55°C
- Optional: Set heat block, incubator, or water bach to 70°C.
- Recommend: 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 300 µL TL Buffer and proceed to Step 3 on the next page.

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 300 µL TL Buffer.

   Optional: For lysis of hair or other tough-to-lyse tissues, a mastermix of TL Buffer and DTT is recommended.
   - 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. Seal plate with sealing film (not provided) and vortex to mix.

4. Incubate at 55°C in a shaking water bath for 3 hours.

   Note: If a shaking water bath is no 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 and pipet up and down several times to mix thoroughly. Let sit at room temperature for 2 minutes.

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

6. Remove seal and carefully transfer 250 µL of the supernatant to a new 96-well deep-well plate (not provided) without disturbing the undigested pellet.

7. Add 290 µL AL Buffer. Vortex for 10 minutes to mix. Proper mixing is crucial for good yield.

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

8. Remove seal from iHDQ Binding Buffer plate and transfer lysate from Step 7 to plate.

   Note: Do not remove seals from remaining reagent plates until ready to load onto instrument in Step 10. Prepare plates according to the "Plasticware Handling and Preparation" section on Page 4.

9. Pipet up and down 5-10 times to mix thoroughly.

10. Start program on magnetic processor. Remove seals from remaining reagent plates and immediately load plates onto instrument according to Plate Layout table on Page 4 when prompted.

    Note: Prepare reagent plates according to the "Plasticware Handling and Preparation" section on Page 4.

11. After run has completed, remove the Elution Buffer Plate from instrument. Seal plate with sealing film or transfer the eluate to a new 1.5 mL tube (not provided) or 96-well microplate (not provided).

12. Store DNA at -20°C.

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

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

Important: Before starting extraction on a magnetic processor, please contact your Omega Bio-tek representative for scripts compatible with your specific instrument.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing-bucket rotor capable of 2,000*g* and adaptor for 96-well plates
- Plate shaker or vortexer with plate adaptor
- Shaking water bath capable of 55°C
- Multichannel pipettes and reagent reservoirs
- 2.2 mL 96-well deep-well plate (Recommend ThermoFisher, Part #95040450 or Thomas Scientific, Part #20A00G709)
- 96-well microplate (Recommend ThermoFisher, Part #97002540)
- 1.5 mL microcentrifuge tubes
- Sealing film (Recommend Cat# AC1200)
- Cold PBS
- 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 each plate according to the "Plasticware Handling and Preparation" section on Page 4.
- Set water bath to 55°C.
- Optional: Set heat block, incubator, or water bach to 70°C.

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 250 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*g* in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 250 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 250 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	290 µL	30.6 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 310 µL AL Buffer/Proteinase K Solution mastermix to cells prepared in Step 1.

4. Pipet up and down to mix thoroughly, or seal plate with sealing film (not provided) and mix by lightly shaking on plate shaker or vortex for 10 minutes. Proper mixing is crucial for good yield.

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 samples every 2-3 minutes.

Optional: Add 5 µL RNase A. Pipet up and down several times to mix thoroughly. Let sit at room temperature for 2 minutes.

6. Remove seal from iHDQ Binding Buffer plate and transfer lysate from Step 5 to plate.

Note: Do not remove seals from remaining reagent plates until ready to load onto instrument in Step 8. Prepare plates according to the "Plasticware Handling and Preparation" section on Page 4.

7. Pipet up and down 5-10 times to mix thoroughly.

8. Start program on magnetic processor. Remove seals from remaining reagent plates and immediately load plates onto instrument according to Plate Layout table on Page 4 when prompted.

   Note: Prepare reagent plates according to the "Plasticware Handling and Preparation" section on Page 4.

9. After run has completed, remove the Elution Buffer Plate from instrument. Seal plate with sealing film or transfer the eluate to a new 1.5 mL tube (not provided) or 96-well microplate (not provided).

10. Store DNA at -20°C.

Mag-Bind® Blood & Tissue HDQ 96 Prefilled Kit - Saliva Protocol

Important: Before starting extraction on a magnetic processor, please contact your Omega Bio-tek representative for scripts compatible with your specific instrument.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of 2,000*g*
- Shaking water bath capable of 55°C
- Vortexer with plate adaptor
- Multichannel pipettes and reagent reservoirs
- 2 mL 96-well deep-well plate (Recommend ThermoFisher, Part #95040450 or Thomas Scientific, Part #20A00G709)
- 96-well microplate (Recommend ThermoFisher, Part #97002540)
- 1.5 mL microcentrifuge tubes
- Sealing film (Recommend Cat# AC1200)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: RNase A (10 mg/mL)

Before Starting:

- Prepare each plate according to the "Plasticware Handling and Preparation" section on Page 4.
- Set shaking water bath to 55°C.
- Optional: Set heat block, incubator, or water bach to 70°C.

1. Centrifuge the saliva tube at 2000*g* for 5 minutes.

2. Transfer 250 µL stabilized saliva sample (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 sample 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.

4. Add 310 µL AL Buffer/Proteinase K Solution mastermix.

5. Pipet up and down to mix thoroughly, or seal plate with sealing film (not provided) and mix by lightly shaking on plate shaker or vortex for 10 minutes. Proper mixing is crucial for good yield.

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

6. 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 7.

Optional: Add 5 µL RNase A. Pipet up and down several times to mix thoroughly. Let sit at room temperature for 2 minutes.

7. Remove seal from iHDQ Binding Buffer plate and transfer lysate from Step 6 to plate.

   Note: Do not remove seals from remaining reagent plates until ready to load onto instrument in Step 9. Prepare plates according to the "Plasticware Handling and Preparation" section on Page 4.

8. Pipet up and down 5-10 times to mix thoroughly.

9. Start program on magnetic processor. Remove seals from remaining reagent plates and immediately load plates onto instrument according to Plate Layout table on Page 4 when prompted.

   Note: Prepare reagent plates according to the "Plasticware Handling and Preparation" section on Page 4.

10. After run has completed, remove the Elution Buffer Plate from instrument. Seal plate with sealing film or transfer the eluate to a new 1.5 mL tube (not provided) or 96-well microplate (not provided).

11. 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	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 PF-HDQ during operation	Contact Omega Bio-tek product support.
	Incorrect plate storage	Store plates in an upright position at room temperature. Examine the plate with Mag-Bind® Particles PF-HDQ carefully before unsealing.
		Before use, centrifuge the reagent plates to ensure that the solutions are in the well and not on the seal.
		Resuspend the Mag-Bind® Particles PF-HDQ by vortexing vigorously to rehydrate dried particles along the sides of the well then centrifuge plate at 2,000*g* for 3 minutes.
	Wells have bubbles	Centrifuge the plates before use.
Problem	**Cause**	**Solution**
Mag-Bind® Particles PF-HDQ do not completely clear from solution	Magnetization time too short	Increase collection time on the magnet.
Problems in downstream applications	Salt carryover	eSPM Buffer must be at room temperature.
	Ethanol carryover	Dry the Mag-Bind® Particles PF-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.

Notes:

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Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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