omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® cfDNA Kit

M3298-00	5 preps
M3298-01	50 preps
M3298-02	200 Preps

Manual Date: July 2025
Revision Number: v9.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

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omegabiotek
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Mag-Bind® cfDNA Kit

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Intended Use

The Mag-Bind® cfDNA Kit is intended for isolation and purification of circulating cell-free DNA (cfDNA) from plasma/serum and urine samples.

The Mag-Bind® cfDNA Kit utilizes magnetic bead-based technology and can be processed either manually or automated on most open-ended liquid handling plateforms as well as magentic processors.

Intended User

The Mag-Bind® cfDNA Kit is intended for professional use and to be used by or under the supervision of professional users, such as laboratory personnel, technicians, researchers and physicians specifically instructed and trained in molecular biology techiniques.

Product Description

The Mag-Bind® cfDNA Kit is designed for rapid and reliable isolation of circulating cell-free DNA from 1-4 mL plasma/serum and urine samples. The Mag-Bind® cfDNA Kit can be processed manually with 15 mL centrifuge tubes or on automated platforms with appropriate plasticware. The procedure eliminates the need for funnels and vacuum steps providing hands-free operation in automated protocols. The uniquely formulated binding buffer from Omega Bio-tek allows for large sample volumes to be processed in automated formats with 4 mL plasma/serum or urine being processed in a 24-well plate. The magnetic properties of the Mag-Bind® Particles CH enable fast magnetic separation, especially during steps involving large volumes. The high-binding capability decreases the amount of magnetic particles required thereby reducing the elution volume; up to 4 mL serum/plasma or urine can be eluted in 50 µL.

This system combines the reversible nucleic acid-binding properties of Mag-Bind® paramagnetic particles with a unique binding system that targets smaller DNA fragments (150-400 bp) and minimizes binding of larger fragments such as genomic DNA.

If the desired target fragment is <150 bp, please see the Optional Protocol Modifications: Recovery of Smaller Fragment section on Page 9 or consult with your Omega Bio-tek representative for a product that will fit your needs.

The purified DNA is of high quality and is suitable for direct use in most downstream applications, such as qPCR and Next Generation Sequencing.

Important:

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

2. 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.

Note: Up to 10 mL sample input volumes can be processed using this kit. Please contact your Omega Bio-tek representative for protocol details.

Kit Contents

Product	M3298-00	M3298-01	M3298-02
Preps	5	50	200
DS Buffer	1.5 mL	20 mL	80 mL
JSB Buffer	25 mL	9 x 25 mL	4 x 220 mL
GT7 Buffer v1.1	11 mL	110mL	2 x 220 mL
SPW Buffer	2.5 mL	25 mL	2 x 50 mL
Elution Buffer	30 mL	250 mL	2 x 250 mL
Proteinase K Solution	350 µL	4 mL	14 mL
Mag-Bind® Particles CH	110 µL	1.1 mL	4.4 mL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® cfDNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CH 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 and away from bright light. 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M3298-00	10 mL
M3298-01	100 mL
M3298-02	200 mL per bottle

2. Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use. The particles must be fully suspended during use to ensure proper binding.

Warnings

This kit is for professional research use.

Please read all instructions carefully before using the kit.

Please decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state/provincial, and/or national regulations. For any assistance, please contact Omega Bio-tek at info@omegabiotek.com.

If you use this kit following an automated extraction workflow, the surface of the automated platform is considered a biohazard. Use appropriate decontamination and disposal methods in adherence to all applicable local state/provincial, and/or national regulations.

Safety Information

All chemicals and biological materials are potentially hazardous.

Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility.

Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Precautions

Some of the buffers included in the Mag-Bind® cfDNA Kit contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions to guanidine containing sample-preparation waste.** Please access the SDSs online for detailed information on the reagents.

Component	Description
DS Buffer 	Contains: Anionic detergent. Danger! Causes serious eye damage. Causes skin irritation. Harmful to aquatic life. Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. If exposed or concerned: call a poison center or doctor/physician. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Take off contaminated clothing and wash before reuse. ON SKIN: Wash with plenty of water and soap. Get medical advice/attention if skin irritation occurs.
Proteinase K Solution 	Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. If exposed or concerned: Call a poison center or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.
JSB Buffer   	Contains: Guanidine thiocyanate and isopropanol. Danger! Flammable liquid and vapor. Causes serious eye damage. Harmful if swallowed. Causes skin irritation. Harmful to aquatic life with long lasting effects. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Keep container tightly closed. Ground/bond container and receiving equipment. Use explosion-proof electrical/ventilating/lighting/intrinsically safe equipment. Use only non-sparking tools. Take precautionary measures against static discharge. Wash all exposed external body areas thoroughly after handling. Do not eat, drink or smoke when using this product. Wear protective gloves, protective clothing, eye protection and face protection. Avoid release to the environment. IN CASE OF FIRE: Use alcohol resistant foam or normal protein foam to extinguish. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call POISON CENTER/doctor/physician/first aider. ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash with plenty of water and soap. Rinse mouth. If skin irritation occurs, get medical advice/attention. Take off contaminated clothing and wash it before reuse.

Precautions

Component	Description
GT7 Buffer v1.1  	Contains: Guanidine thiocyanate. Danger! Harmful if swallowed. Causes severe skin burns and eye damage. Do not breathe mist/vapors/spray. Harmful to aquatic life with long lasting effects. Wear protective clothing, eye protection and face protection. Wash all exposed external body areas thoroughly after handling. Do not eat, drink or smoke when using this product. Avoid release to the environment. SWALLOWED: Rinse mouth. Do NOT induce vomiting. Call a POISON CENTER/doctor/physician/first aider/ if you feel unwell. ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse. IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor/physician/first aider. INHALED: Remove person to fresh air and keep comfortable for breathing.

Recovery of Smaller Fragments (<150 bp)

The standard protocol can be modified by either addition of supplemental JSB Buffer or by addition of a combination of JSB Buffer and isopropanol depending on the fragment size of interest. Refer to Table 1 and Figure 1 below to determine which protocol to use for recovery of smaller fragments.

Modified Protocol 2 will require additional JSB Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative for details.

Table 1: Additional JSB and Isopropanol for small fragment recovery.
The table below describes the total amount of JSB Buffer and/or 100% isopropanol used for the recovery of desired small fragments. Additional JSB and/or isopropanol volumes are in relation to starting volume of serum/plasma. For example, for Modified Protocol 1 using 1 mL serum/plasma, add 1 mL JSB Buffer and 1 mL 100% isopropanol. Modified Protocol 1 should be used to recover fragments as small as 50 bp; however, decreased yields of fragments >150 bp are observed with this modification compared to the standard protocol.

Protocol Modifications	Recovered Fragment Size (bp)	Total Reagents Used	
		JSB Buffer	100% Isopropanol
Standard Protocol	> 150 bp	1 volume	------
Modifed Protocol 1	> 50 bp	1 volume	1 volume
Modifed Protocol 2	> 75 bp	2 volumes	------

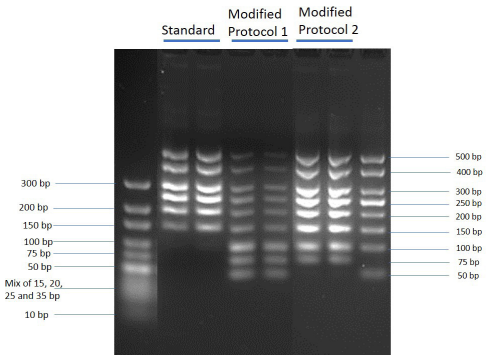

Figure 1: Fragment Sizes Recovered with Different Protocols.
Gel image depicting the recovery of short DNA fragments using different protocol modifications. 10 µL of the eluted DNA was run on a 3% agarose gel for 1 hour at 100V to analyze the size fragments recovered with each of those protocols.

Guidelines for cfDNA Quantification

DNA quantification is typically done by spectrophotometric-based (NanoDrop®) or fluorometric-based methods (Qubit®). Both of these methods are inaccurate when it comes to quantifying circulating, cell-free DNA because cfDNA is usually present in low amounts and these methods are unable to distinguish between cfDNA and high molecular weight cellular genomic DNA. It is important to establish accurate strategies to not only precisely quantify cfDNA but also to draw pertinent conclusions about the extraction efficiency. Some of the strategies that can aid in quantification of cfDNA are elucidated below.

Tape Station

The Cell-free DNA ScreenTape assay for TapeStation systems provides accurate sizing and quantification of cfDNA, as well as DNA quality assessment with %cfDNA information. The %cfDNA is indicative of the percentage of cfDNA compared to the genomic DNA in the purified sample.

qPCR

Quantification based on qPCR analysis is effective if the primers are targeting just the cfDNA fraction and not the gDNA fraction. If not, the primers are going to amplify from both the cfDNA and gDNA fractions present in the eluate skewing the results. For example, use of tumor-specific primers if the cfDNA is tumor-derived can analyze the cfDNA fraction without the gDNA interference. For kit evaluation purposes, using a spike in such as 200 bp sheared bacterial DNA in plasma/serum along with bacterial specific primers can offer information about the extraction efficiency in terms of actual cfDNA present in the total DNA isolated.

cfDNA integrity analysis

cfDNA integrity analysis is done by real-time PCR of ALU-repeats using two sets of primers to amplify different lengths of DNA fragments (115 bp and 247 bp). ALU sequences are highly abundant in the human genome and amplification of the 115-bp ALU amplicon represents the total amount of DNA fragments (both short and long fragments) whereas the 247-bp ALU amplicon primarily reflects the amount of long DNA fragments. cfDNA integrity can be reported as integrity index, which is calculated as the ratio of ALU247 to ALU115. If the isolated DNA is mainly gDNA, ALU247/ALU115 is expected to be 1. The ratio is between 0 to 1 if short fragments (cfDNA) are present. Typically, the higher the amount of cfDNA in the sample, the higher the integrity index.

Protocol for 1 mL Serum/Plasma

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:

- Magnetic separation device for 1.5/2.0 mL microcentrifuge tubes
- Incubator capable of 60°C
- Shaker or rocker for Step 8
- Vortexer
- 15 mL centrifuge tubes
- 1.5 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- Optional: microplate for DNA storage

Before Starting:

- Prepare SPW Buffer according to the "Preparing Reagents" section on Page 5.
- Set incubator to 60°C.
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use.

1. Add 500-1000 µL serum/plasma samples to a 15 mL centrifuge tube (not provided). Bring the volume up to 1 mL with Elution Buffer if sample volume is less than 1 mL.

2. Add 15 µL Proteinase K Solution.

3. Add 67 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 20 minutes. Mix by inverting or shaking every 10 minutes.

6. Let sit at room temperature for 10 minutes.

Optional: If desired fragment size is <150 bp, refer to Optional Protocol Modifications: Recovery of Smaller Fragments on Page 7 before proceeding to Step 7. Modified Protocol 2 will require additional JSB Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative for details.

7. Add 1 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

8. Add 5 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Let sit for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

9. Transfer 1 mL lysate to a 1.5 mL microcentrifuge tube (not provided).

10. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

12. Transfer the remaining lysate from Step 8 to the 1.5 mL microcentrifuge tube used in the previous steps.

13. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

15. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

16. Add 500 µL GT7 Buffer v1.1.

17. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH.

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

18. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

    Note: GT7 Buffer v1.1 may foam during vortexing. Remove foam from cap then remove supernatant.

20. Repeat Steps 15-19 for a second GT7 Buffer v1.1 step.

21. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

22. Add 500 µL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

23. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH.

24. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

26. Repeat Steps 21-25 for a second SPW Buffer step.

27. Remove the tube from the magnetic separation device for approximately 30 seconds.

28. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH.

29. Aspirate and discard the residual SPW Buffer.

30. Leave the tube on the magnetic separation device for 25 minutes to dry the Mag-Bind® Particles CH.

31. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

32. Add 30-60 µL Elution Buffer.

33. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.

34. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

35. Transfer the cleared supernatant containing purified DNA to a 1.5 mL microcentrifuge tube or clean microplate (not provided).

36. Store DNA at -20°C.

Protocol for 2 mL Serum/Plasma

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:

- Magnetic separation device for 15 mL centrifuge tubes and 1.5/2.0 mL microcentrifuge tubes
- Incubator capable of 60°C
- Shaker or rocker for Step 8
- Vortexer
- 15 mL centrifuge tubes compatible with magnetic separation device used
- 1.5 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- Optional: microplate for DNA storage

Before Starting:

- Prepare SPW Buffer according to the "Preparing Reagents" section on Page 5.
- Set incubator to 60°C.
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use.

1. Add up to 2 mL serum/plasma samples to a 15 mL centrifuge tube (not provided). Bring the volume up to 2 mL with Elution Buffer if sample volume is less than 2 mL.

2. Add 30 µL Proteinase K Solution.

3. Add 135 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 25 minutes. Mix by inverting or shaking every 10 minutes.

6. Let sit at room temperature for 10 minutes.

Optional: If desired fragment size is <150 bp, refer to Optional Protocol Modifications: Recovery of Smaller Fragments on Page 7 before proceeding to Step 7. Modified Protocol 2 will require additional JSB Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative for details.

7. Add 2 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

8. Add 10 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Let sit for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

9. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

11. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

12. Add 1 mL GT7 Buffer v1.1.

13. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH.

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

14. Transfer the resuspended Mag-Bind Particles CH to a new 1.5 mL centrifuge tube (not provided). Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure.

15. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

17. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

18. Add another 1 mL GT7 Buffer v1.1.

19. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH.

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

20. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

22. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

23. Add 1 mL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

24. Vortex for 2 minutes to resuspend the Mag-Bind® Particles CH.

25. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

27. Repeat Steps 22-26 for a second SPW Buffer step.

28. Remove the tube from the magnetic separation device for approximately 30 seconds.

29. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH.

30. Aspirate and discard the residual SPW Buffer.

31. Leave the tube on the magnetic separation device for 25 minutes to dry the Mag-Bind® Particles CH.

32. Remove the tube containing the Mag-Bind® Particles CH from the magnetic separation device.

33. Add 50-100 µL Elution Buffer.

34. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.

35. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

36. Transfer the cleared supernatant containing purified DNA to a 1.5 mL microcentrifuge tube or clean microplate (not provided).

37. Store DNA at -20°C.

Protocol for 4 mL Serum/Plasma

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:

- Magnetic separation device for 24-well deep-well plates (Alpaqua Magnum FLX24) or for 15 mL centrifuge tubes and 1.5/2.0 mL microcentrifuge tubes
- Incubator capable of 60°C
- Shaker or rocker for Step 8
- Vortexer
- 24-well deep-well plate or 15 mL centrifuge tubes compatible with magnetic separation device used
- 1.5 mL microcentrifuge tubes compatible with magnetic separation device used
- 100% ethanol
- Optional: microplate for DNA storage

Before Starting:

- Prepare SPW Buffer according to the "Preparing Reagents" section on Page 5.
- Set incubator to 60°C.
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use.

1. Add up to 4 mL serum/plasma samples to a 15 mL centrifuge tube or 24-well deep-well plate (not provided). Choose the correct plasticware depending on the magnetic separation device being used. Bring volume up to 4 mL with Elution Buffer if the volume of sample is less than 4 mL.

2. Add 60 µL Proteinase K Solution.

3. Add 270 µL DS Buffer.

4. Vortex at maximum speed or pipet up and down to mix thoroughly.

5. Incubate at 60°C for 30 minutes. Mix by inverting or shaking every 10 minutes.

6. Let sit at room temperature for 10 minutes.

Optional: If desired fragment size is <150 bp, refer to Optional Protocol Modifications: Recovery of Smaller Fragments on Page 7 before proceeding to Step 7. Modified Protocol 2 will require additional JSB Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative for details.

7. Add 4 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

8. Add 20 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Let sit for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

9. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

11. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

12. Add 1 mL GT7 Buffer v1.1.

13. Vortex for 5 minutes to resuspend the Mag-Bind® Particles CH.

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

14. Transfer the resuspended Mag-Bind® Particles CH to a new 1.5 mL centrifuge tube (not provided) if using a 15 mL centrifuge tube for Steps 1-13. Use a magnetic separation device designed for 1.5/2.0 mL tubes for the remaining procedure. If using a 24-well deep-well plate for Steps 1-13, continue to use the 24-well deep-well plate and a 24-well magnet.

15. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

17. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

18. Add another 1 mL GT7 Buffer v1.1.

19. Vortex for 5 minutes to resuspend the Mag-Bind® Particles CH.

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

20. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

22. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

23. Add 1 mL SPW Buffer.

    Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

24. Vortex for 5 minutes to resuspend the Mag-Bind® Particles CH.

25. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

27. Repeat steps 22-26 for a second SPW Buffer step.

28. Remove the tube/plate from the magnetic separation device for approximately 30 seconds.

29. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH.

30. Aspirate and discard the residual SPW Buffer.

31. Leave the tube/plate on the magnetic separation device for 25 minutes to dry the Mag-Bind® Particles CH.

32. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

33. Add 50-100 µL Elution Buffer.

34. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.

35. Place the tube on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

36. Transfer the cleared supernatant containing purified DNA to a 1.5 mL microcentrifuge tube or clean microplate (not provided).

37. Store DNA at -20°C.

Protocol for 4 mL Urine

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:

- Magnetic separation device for 24-well deep-well plates (Alpaqua Magnum FLX24)
- Magnetic separation device for 96-well deep-well plates
- Magnetic separation device for 15 mL centrifuge tubes and 1.5/2.0 mL microcentrifuge tubes
- Shaker or rocker for Step 7
- Vortexer
- 24-well deep-well plate and 96-well deep well plate compatible with magnetic separation device used
- 15 mL centrifuge tubes and 1.5 mL/2.0 mL microcentrifuge tubes compatible with magnetic separation device used
- 96-well microplate
- 100% ethanol
- 70% ethanol
- 100% isopropanol
- 1X PBS
- **Required:** Mag-Bind® TotalPure NGS (M1378)

Before Starting:

- Shake or vortex the Mag-Bind® Particles CH and Mag-Bind® TotalPure NGS (not provided) to fully resuspend the particles before use.
- Allow Mag-Bind® TotalPure NGS to come to room temperature before use.
- Prepare 70% ethanol.

1. Obtain urine samples (25-100 mL). Use either fresh urine or urine preserved in stabilization media such as Streck Urine Preserve or the DNA Genotek's Colli-Pee™ UAS™.

2. Centrifuge samples at 3,000-4,000*g* for 15 minutes.

3. Transfer 4 mL urine supernatant into 15 mL centrifuge tube (not provided) or 24-well deep well plate (not provided). Choose the correct plasticware depending on the magnetic separation device being used. Bring volume up to 4 mL with 1X PBS if the volume of sample is less than 4 mL.

4. Add 270 µL DS Buffer.

5. Vortex at maximum speed, pipet mix up and down, or invert the tube to mix thoroughly.

6. Add 4 mL JSB Buffer. Vortex at maximum speed for 30 seconds or pipet up and down to mix thoroughly.

7. Add 20 µL Mag-Bind® Particles CH. Invert the sample 10 times or pipet up and down to mix. Let sit for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10 minute incubation period by shaking or rocking. ***Do not vortex at high speeds*** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Mag-Bind® Particles CH resuspended in solution.

8. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

10. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

11. Add 1 mL GT7 Buffer v1.1.

12. Vortex for 5 minutes to resuspend the Mag-Bind® Particles CH.

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

13. Transfer the resuspended Mag-Bind® Particles CH to a new 1.5 mL/2.0 mL microcentrifuge tube (not provided) or new 96-well deep-well plate (not provided).

    Note: If using a 15 mL centrifuge tube for Steps 1-12, use a magnetic separation device designed for 1.5 mL/2.0 mL microcentrifuge tubes for the remaining of the procedure. If using a 24-well deep-well plate for Steps 1-12, change to use the 96-well deep-well plate and a 96-well magnet for the remaining of the procedure.

14. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

16. Leave the tube/plate on the magnetic separation device for 5 minutes to dry the Mag-Bind® Particles CH.

17. Remove the tube/plate containing the Mag-Bind® Particles CH from the magnetic separation device.

18. Add 50-100 µL Elution Buffer.

19. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® Particles CH.

20. Place the tube/plate on the magnetic separation device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

21. Transfer the cleared supernatant containing cfDNA to a new 1.5 mL microcentrifuge tube or 96-well microplate (not provided).

22. Add 2 volumes of Mag-Bind® TotalPure NGS and 3 volumes of 100% isopropanol (not provided) to the tube/plate containing cfDNA in Step 21.

    Note: If sample volume is 100 µL, add 200 µL Mag-Bind® TotalPure NGS and 300 µL 100% isopropanol.

23. Vortex for 1 minute to mix thoroughly.

24. Let sit at room temperature for 5 minutes.

25. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® TotalPure NGS. Let sit at room temperature until the Mag-Bind® TotalPure NGS is completely cleared from solution.

26. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® TotalPure NGS.

27. Remove the tube/plate containing the Mag-Bind® TotalPure NGS from the magnetic separation device.

28. Add 500 µL 70% ethanol (not provided).

29. Vortex for 1 minute.

30. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® TotalPure NGS. Let sit at room temperature until the Mag-Bind® TotalPure NGS is completely cleared from solution.

31. Repeat Steps 26-30 for a second 70% ethanol wash step.

32. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® TotalPure NGS.

    Note: It is important to dry the Mag-Bind® TotalPure NGS before elution. Residual ethanol may interfere with downstream applications.

33. Leave the tube/plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® TotalPure NGS. Remove any residual liquid with a pipettor.

34. Remove the tube/plate from the magnetic separation device.

35. Add 20-50 µL Elution Buffer.

36. Vortex at room temperature for 5 minutes to resuspend the Mag-Bind® TotalPure NGS.

37. Place the tube/plate on a magnetic separation device to magnetize the Mag-Bind® TotalPure NGS. Let sit at room temperature until the Mag-Bind® TotalPure NGS is completely cleared from solution.

38. Transfer the cleared supernatant containing purified cfDNA to a new 1.5 mL microcentrifuge or 96-well microplate.

39. Store cfDNA at -20°C.

Contact Information

To reorder supplies, report a device failure or complaint, please contact:

	**Manufacturer** Omega Bio-tek, Inc. 400 Pinnacle Way Suite #450 Norcross, GA 30071, USA Website: www.omegabiotek.com Email: info@omegabiotek.com SRN: US-MF-000024148

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Picture	Description
	Use-by date
	Check components for storage conditions
	Lot number
	Manufacturer
	No additional hazards or not classified as hazardous according to GHS
	Website
	Telephone
	Fax
	Email
	LinkedIn
	Twitter
	Facebook

Document Revision History

Revision	Description
v9.0, July 2025	A new protocol for the isolation of circulating DNA from urine sample has been added. The protocol has been updated to new template.
v8.5, November 2024	Guidelines for cfDNA Quantification has been updated.
v8.4, April 2024	Warnings and Safety Information has been added.
v8.3, May 2023	JSB Buffer in kit M3298-01 has been updated to be provided in 9 individual bottles instead of one bulk bottle. This is to comply with the primary package volume requirements for the shipment of flammable liquids.
v8.0, January 2021	Mag-Bind® Particles CH used in the extraction has been reduced to mitigate inhibition and improve downstream performance.
v7.0, June 2020	GT7 Buffer has been renamed GT7 Buffer v1.1. The buffer formula has been altered to improve stability.
v5.0, September 2018	A protocol modification for fragments smaller than 150 bp has been added. GT7 Wash Buffer has been renamed GT7 Buffer. SPW Wash Buffer has been renamed SPW Buffer. The buffer formula for both has not changed.
v4.0, March 2018	A new protocol for the isolation of circulating DNA from a 2 mL serum/plasma sample has been added. Mag-Bind® Particles CH drying times prior to elution has been updated.
v3.0, October 2017	A new protocol for the isolation of circulating DNA from a 4 mL serum/plama sample has been added.
v1.0, February 2017	Initial Release.

Notices & Disclaimers

For European Union Use.

JSB Buffer and GT7 Buffer v1.1 contain 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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Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071

www.omegabiotek.com

770-931-8400

770-931-0230

info@omegabiotek.com

omega-bio-tek

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