






MB Fit24™ cfDNA Kit




Product	Preps
B3298-10-48PF	48 preps

Manual Date: December 2023
Revision Number: v1.0

For Research Use Only

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MB Fit24™ cfDNA Kit

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Intended Use

For Professional Research use only.

The MB Fit24™ cfDNA Kit is intended for use in a laboratory setting performing cfDNA extractions from up to 4 mL plasma/serum on the MagBinder® Fit24 Nucleic Acid Purification System.

Intended User

This kit is intended for professional use.

The MB Fit24™ 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 techniques and/or operating magnetic processor platforms.

Product Description

The MB Fit24™ cfDNA Kit is designed for rapid and reliable isolation of circulating DNA from up to 4 mL plasma/serum samples with the use of the MagBinder® Fit²⁴ Nucleic Acid Purification System. This kit is automation-ready with Mag-Bind® cfDNA Kit reagents arrayed into a ready-to-use reagent cartridge specifically configured for the MagBinder® Fit²⁴ instrument to provide faster and consistent results. This procedure provides a semi-automated extraction workflow for processing up to 24 samples in less than 55 minutes once loaded onto the MagBinder® Fit²⁴.

The MB Fit24™ cfDNA Kit enhances ease of use, convenience, and extraction accuracy and reduces hands-on time by skipping reagent preparation and buffer dispensing steps. The samples are lysed offline, and lysate is transferred to the reagent well containing the binding buffer. The uniquely formulated binding buffer allows for large sample volumes to be processed in semi-automated format with up to 4 mL plasma or serum being processed in one reagent cartridge without sample splitting. The magnetic properties of the Mag-Bind® Particles CH enable fast magnetic separation, especially during steps involving large volumes. The high-binding capacity decreases the amount of magnetic particles required thereby reducing the elution volume. Up to 4 mL plasma or serum can be eluted in as little as 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. The purified cfDNA is of high-quality and is suitable for direct use in most downstream applications such as PCR, digital PCR, next generation sequencing, etc.

The MagBinder® Fit²⁴ instrument is preprogrammed with purification protocols that are validated to work with prefilled reagent cartridges. The instrument requires the user to select the appropriate protocol depending on the kit being used. If using the MB Fit24™ cfDNA Kit for sample volumes other than those listed in this manual, please contact your Omega Bio-tek representative for preprocessing instructions.

Kit Contents

Product	B3298-10-48PF
Purifications	48
Elution Tube (2 mL)	50
MagBinder® Tip Comb	2 x 2 combs
Prefilled Reagent Cartridge*	48
DS Buffer	20 mL
Elution Buffer	250 mL
Mag-Bind® Particles CH	1.1 mL
Proteinase K Solution	4 mL

*Buffers and their location in the prefilled reagent cartridges are shown on Page 9.

Storage and Stability

All of the MB Fit24™ cfDNA 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 recommended temperatures as mentioned on the label and away from bright light. Once product is opened, continue to maintain the product in accordance with labeled instructions. Ensure that caps are properly tightened following each use. 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.

Warnings

This kit is for professional 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 www.omegabiotek.com.

After extraction, the surface of the MagBinder® Fit²⁴ 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 reagents 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 MB Fit24™ 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	<p>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.</p>
eSPW Buffer	<p>Contains: Ethanol. Danger! Highly flammable liquid and vapor. Causes serious eye damage. 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. Wear protective gloves, protective clothing, eye protection, and face protection. 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 so. Continue rinsing. If eye irritation persists, get medical advice/attention. ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.</p>

Quantification

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.

TapeStation or Fragment Analyzer

Fragment size profiling can be used for cfDNA quantification. cfDNA are usually small fragments of DNA with a size distribution peak at ~170 bp. The peak heights and separation on the electropherogram corresponding to cfDNA fragment size and gDNA size can shed light on the relative proportions of each and can help draw conclusions about cfDNA extraction efficiency. The regional analysis functionality offered by the software can further assist in approximating the cfDNA concentration. For example, DNA concentration within the 100-300 bp region where cfDNA is most likely to be present can be quantified using the TapeStation software using this functionality.

qPCR

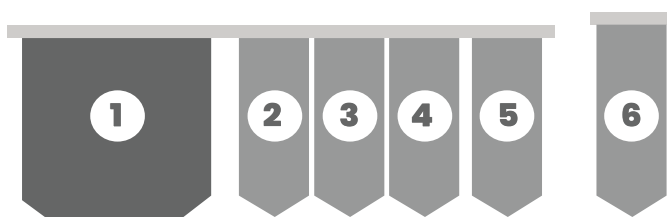
Quantification based on qPCR analysis is effective if the primers are targeting just the cfDNA fraction and not the gDNA fraction. If not, the primers are going to amplify from both the cfDNA and gDNA fractions present in the eluate skewing the results. For example, use of tumor-specific primers if the 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.

Plasticware Handling and Preparation

1. Always check reagent cartridges for presence of precipitation before starting extraction. Dissolve precipitates by warming the reagent cartridge at 37°C with gentle shaking.
2. Flick downward or gently tap each cartridge before removing the seal to ensure reagents are in the bottom of the wells and not clinging to the underside of the seal.
3. Carefully remove seal from the reagent cartridges and immediately place the cartridge on loading tray when ready.
4. The table below details contents of reagent cartridge.



Well Position	Content	Volume per well
1	JSB Buffer	4 mL
2	GT7 Buffer v1.1	1 mL
3	GT7 Buffer v1.1	1 mL
4	eSPW Buffer	1 mL
5	eSPW Buffer	1 mL
6	Elution Buffer ¹	50 µL – 100 µL

¹Elution Buffer must be added to the Elution Tube prior to starting extraction.

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Protocol for up to 4 mL Plasma/Serum

Important: When starting program on the MagBinder® Fit24 instrument, make sure that the correct and current version of the protocol, **OBTRB3298**, is selected.

Materials and Equipment to be Supplied by User:

- Incubator or heat block capable of 60°C
- Vortexer
- Serological pipette capable of 10 mL
- 15 mL centrifuge tubes

Before Starting:

- Prepare reagent cartridges according to the “Plasticware Handling and Preparation” on Page 9.
 - Set incubator or heat block to 60°C.
1. Add up to 4 mL plasma/serum sample to a 15 mL centrifuge tube (not provided). 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.

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7. Remove seal from reagent cartridge and place into the MagBinder® Fit²⁴ loading tray. Transfer lysate from Step 6 to Well 1 of the reagent cartridge. Pipet up and down 5-10 times to mix thoroughly.

Note: Prepare reagent cartridge according to the “Plasticware Handling and Preparation” on Page 9.

8. Add 20 µL Mag-Bind® Particles CH to Well 1 of the reagent cartridge. Pipet up and down to mix thoroughly.

9. Prepare Elution Tube by filling with 50 µL - 100 µL Elution Buffer volume.

10. Load the tip combs on the tip comb holder.

Note: Ensure the tip comb is pushed all the way back and completely in place.

11. Place loading tray containing reagent cartridges and Elution Tubes onto the instrument deck. Gently press down on the reagent cartridges and Elution tubes so they are secure on the deck. Slide the deck into the instrument and close the door.

Note: Ensure the Elution Tubes are positioned open with caps oriented to the right of the tube. The orientation of the Elution Tubes is important in preventing an instrument error during the run.

12. Start the program on the MagBinder® Fit²⁴ instrument.

13. Once run has completed, remove the Elution Tube from instrument and cap tightly.

14. 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	Insufficient amount of Mag-Bind® Particles CH Beads was added	Vortex the tube containing the magnetic particles thoroughly immediately before use.
	Loss of Mag-Bind® Particles CH during operation	Contact Omega Bio-tek product support.
	Incorrect plasticware used during operation	Make sure to use plasticware approved for MagBinder® Fit ²⁴ . Contact Omega Bio-tek product support.
	Incorrect reagent cartridge storage	Store reagent cartridges in an upright position at room temperature.
Flick downward or gently tap each reagent cartridge before removing the seal to ensure reagents are in the bottom of the wells and not clinging to the underside of the seal.		
Problems in downstream applications	Ethanol carryover	Dry the Mag-Bind® Particles CH before elution. Contact Omega Bio-tek product support for protocol adjustments.
Mag-Bind® Particles CH do not completely clear from solution	Magnetization time too short	Increase collection time on the magnet.
	Magnetic particles present in eluate	Place Elution Tube on magnetic stand, wait for particles to pellet and then transfer sample to another tube.
Problems with instrument operation	Wrong method run on the MagBinder® Fit ²⁴ Instrument	Check that the correct method was run.
	Instrument errors at start of operation	Make sure the reagent cartridges are nested securely on the deck and tip combs are secured in tip holder.
		Refer to the MagBinder® Fit ²⁴ instrument manual for error code description.

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 Authorization 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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Qiagen®, QIAvac® and Vacman® are all trademarks of their respective companies.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.


Symbols

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

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

Contact Information

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

 The logo for Omega Bio-tek, Inc. is a stylized black silhouette of a factory with three buildings of varying heights and a tall chimney on the right side.	<p>Manufacturer Omega Bio-tek, Inc. 400 Pinnacle Way Suite #450 Norcross, GA 30071, USA Website: www.omegabiotek.com Email: info@omegabiotek.com</p>
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Revision History

Revision	Description
v1.0, December 2023	Initial release

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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