MAGBIO

Liquid Biopsy Research - "We are where you start"

HighPrep™ FFPE Tissue DNA Kit

Catalog Nos. HPFF-D25, HPFF-D96, HPFF-D96X4
Manual Revision v5.03

- Genomic DNA isolation from FFPE (**F**ormalin-**F**ixed, **P**araffin-**E**mbedded) Tissues
- Proprietary deparifinization reagent. Safer than xylene and other toxic solvents.
- Magnetic beads based chemistry
- Adaptable to a liquid handling workstation

PROTOCOL

Contents

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ FFPE Tissue DNA Kit is a high quality, high throughput genomic purification of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Instead of using xylene, the kit utilizes a proprietary deparaffinization reagent for safe and convenient paraffin removal while eliminating the needs of centrifugation or changing tubes. To overcome cross linking of nucleic acids caused by formalin fixation, the kit uses specially formulated buffers and lysing conditions to release DNA from tissue sections. Utilizing magnetic bead purification technology, the kit is high-throughput capable and can be adapted to most liquid handling workstations in the market.

Fragmentation of Nucleic Acids Consideration

The purified genomic DNA is suitable for downstream applications such as quantitative real-time RT-PCR, sequencing and mutation screening. Because tissue fixation and embedding causes significant fragmentation (nucleic acid modifications), DNA recovered from FFPE samples exhibits broad size distribution and is not recommended for downstream applications that require full length DNA.

Workflow Overview

The HighPrep™ FFPE DNA Kit utilizes magnetic beads technology to bind to the DNA, therefore a magnetic processing plate is required. Samples are first treated with the FFPE DP Reagent (deparaffinization reagent). Next, the samples are lysed in FFPE DTL Buffer following digestion with Pro K Solution. The lysate is then heated to denature the proteinase. FFPE DB Buffer and the MAG-D3 magnetic particles are added to bind the nucleic acid. The DNA bound beads are washed twice and eluted with Elution Buffer or nuclease-free water.

Kit Contents and Storage

HighPrep™ FFPE Tissue DNA Kit Catalog No.	**HPFF-D25**	**HPFF-D96**	**HPFF-D96X4**	**STORAGE**
Number of Preps	**25**	**96**	**384**	
FFPE DP Reagent	15 mL	50 mL	200 ml	15-25°C
FFPE DTL Buffer	6 mL	20 mL	80 ml	15-25°C
FFPE DB Buffer	6 mL	20 mL	80 ml	15-25°C
FFPE DW1 Buffer[1]	8 mL	30 mL	120 ml	15-25°C
FFPE DW2 Buffer[1]	8 mL	25 mL	100 ml	15-25°C
Pro K Solution[2]	600 µL	2 mL	8 ml	2-8°C
Elution Buffer	2 mL	10 mL	40 ml	15-25°C
LPA	300 µL	1 mL	4 ml	2-8°C
MAG-D3 Particles	300 µL	1 mL	4 ml	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 12 months when stored accordingly.
[2]Pro K Solution comes in a ready to use solution. Component is stable for 1 year when stored at 15-25°C. For storage longer than 1 year, storage at 2-8°C is recommended.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 96-100% Ethanol	Storage
HPFF-D25	FFPE DW1 Buffer	8 mL	Room Temp 15-25°C
	FFPE DW2 Buffer	32 mL	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Catalog No.	Component	Add 96-100% Ethanol	Storage
HPFF-D96	FFPE DW1 Buffer	30 mL	Room Temp 15-25°C
	FFPE DW2 Buffer	100 mL	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Catalog No.	Component	Add 96-100% Ethanol	Storage
HPFF-D96X4	FFPE DW1 Buffer	120 mL	Room Temp 15-25°C
	FFPE DW2 Buffer	400 mL	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Protocol: FFPE Tissue DNA Kit - microcentrifuge tube or plate format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 1.5 ml microcentrifuge tube or 2 ml 96 well round-bottom plates
- ☐ Water bath or heat block capable of 55°C
- ☐ Water bath or heat block capable of 65°C
- ☐ Sealing film (for plate format)
- ☐ 100% Isopropanol
- ☐ 96-100% Ethanol
- ☐ Magnetic separation device for 96-well plate (Suggestion: Alpaqua Magnum EX) or
- ☐ if using 1.5 ml microcentrifuge tube, use applicable magnetic rack stand.
  Optional: 10 mg/ml RNase A (when RNA-free genomic DNA is required)

Things to do before starting

- ☐ Ensure FFPE DW1 Buffer and FFPE DW2 Buffer are prepared according to the instructions on page 2 and are at room temperature.
- ☐ Warm up Elution Buffer to 65°C.
- ☐ Set water bath or heat block to 55°C and 65°C.
- ☐ Resuspend the MAG-D3 solution completely by vortexing.

Protocol

1. **Add 500 µl FFPE DP Reagent into a well of a microcentrifuge tube or 96 well round bottom plate.**

2. **Cut 2-3 paraffin sample sections between 5-10 µm thickness (up to 15 mg) and add to the tube/well containing the FFPE DP Reagent. Vortex for 20 seconds to mix thoroughly.**

3. **Incubate at 65°C for 15 min. Vortex briefly once during incubation.**

4. **Add 200 µl FFPE DTL Buffer and 20 µl Pro K Solution and vortex to mix thoroughly.**

5. **Incubate at 55°C for 3 hours. Vortex briefly once during incubation.**
   Note: Incubation can proceed overnight.

6. **Add 200 µl FFPE DB Buffer. Incubate at room temperature for 10 min, briefly vortex the tube/plate several times during incubation.**
   Optional: If RNA-free gDNA is required, add 10 µl RNase A (10 mg/mL, not included) and incubate for 5 min.

7. **Add 300 µl isopropanol and 10 µl MAG-D3 particles to the sample. Vortex at maximum speed for 2 minutes. Incubate at room temperature for 10 min.**
   Note: If expecting low DNA content from sample, add 10 µl LPA (included with kit).
   ⚠ Shake thoroughly the MAG-D3 particles to fully resuspend before use.

8. **Place the sample tube on the magnetic separation device until the beads completely clear the solution.** There will be two layers in the tube. An upper pink color layer containing the paraffin and a lower aqueous phase layer.

9. **Aspirate and discard all supernatant.** Do not disturb the magnetized beads.

10. **Remove the tube/plate from the magnetic separation device.**

11. **Add 600 µl FFPE DW1 Buffer to the sample. Resuspend the beads by vortexing at maximum speed for 1 min (20 sec increments)or by pipetting up and down 20 times.**
    Note: Complete resuspension of the magnetic particles is critical for obtaining high purity DNA. FFPE DW1 Buffer must be diliuted with ethanol prior to use. See "Preparation of Reagents" on page 2 for FFPE DW1 Buffer.

12. **Place the sample tube/plate on the magnetic separation device until the beads clear the solution.**

13. **Aspirate and discard the cleared supernatant.** Do not disturb the magnetized beads.

14. **Remove the tube/plate from the magnetic separation device.**

15. **Add 600 µl of FFPE DW2 Buffer to the sample. Resuspend the beads by vortexing at maximum speed for 1 min (20 sec increments) or by pipetting up and down 20 times.**
    * FFPE DW2 Buffer must be diliuted with ethanol prior to use. See "Preparation of Reagents" on page 2 for FFPE DW2 Buffer.

16. **Place the sample plate on the magnetic separation device until the beads clear the solution.**

17. **Aspirate and discard the cleared supernatant.** Do not disturb the magnetized beads.

18. **Repeat steps 14-17 for another wash .**

19. **Leave the tube on the magnetic separation device for 5-10 min to air dry the magnetized beads.** It is critical to completely remove all liquid from each well since ethanol carryover in the eluate may interfere with some downstream applications. Do not over dry the beads.

20. **Add 30-50 µl Elution Buffer or nuclease-free water preheated to 65°C to the sample. Resuspend the beads by vortexting at maximum speed for 1 min or by pipetting up and down 20 times.**

21. **Incubate sample at room temperature for 10 min.**
    Note: Incubating at 65°C may improve yield.

22. **Place the sample tube/plate on the magnetic separation device until the beads completely clear the solution.**

23. Pipet the cleared supernatant containing purified DNA to a new 1.5 ml microcentrifuge tube or plate. Store the DNA at -20°C.

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (within US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA Yields	Tissue was not digested.	Ensure that the tissue is fully submerged during the digestion steps.
	Incomplete resuspension of MAG-D3 particles.	Resuspend MAG-D3 particles by pipette mixing or vortexing vigorously before use.
	Loss of MAG-D3 particles during operation.	Avoid disturbing the MAG-D3 particles during aspiration of supernatant.
	FFPE DW1 Buffer and/or FFPE DW2 Buffer may not be prepared correctly.	Prepare buffers accordingly. See "Preparation of Reagents" on page 2.
	Binding was incomplete.	Ensure that the samples are mixed well before collecting the beads. Increase pipette mixing or vortex/shake plate to ensure complete mixing.
MAG-D3 particles do not completely clear from solution	Too short of magnetizing time.	Increase collection time on the magnet.
Problems in downstream applications	DNA is over fixated during tissue formalin fixation.	Extend incubation time at 90°C to 90 min.
Carryover of the magnetic beads in the elution	Bead collection time is too short.	Increase bead collection time. Residual magnetic particles in eluted DNA will not affect downstream application. Residual magnetic particles in eluted DNA can be magnetized again and the DNA eluate can be transfered to a new storage plate.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ FFPE Tissue DNA Kit (96)	HPFF-D96	96
HighPrep™ FFPE Tissue DNA KIt 384 (96 x 4)	HPFF-D96X4	384

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