Clean FFPE DNA/RNA Kit

Catalog Numbers:
CFFPE-DR0096: 96 preps
CFFPE-DR0384: 384 preps

Batch No: See package

Shipping: Room temperature

Storage and stability: CleanNA Particles CFFPE should be stored at 4°C and DNase Digestion Buffer, DNase I and RNA Rebind Buffer should be stored at -20°C upon receipt, store all other components at room temperature. See page 3 for more storage information.

Intended use: Clean FFPE is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

USER MANUAL

Manual revision v5.00

Quality Control: Each lot of Clean FFPE is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at info@cleanna.com or +31 (0) 182 22 33 50.

Safety precautions: When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.

Emergency: In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or info@cleanna.com.

Expiry: When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

FOR RESEARCH USE ONLY

Contents

Introduction and Principle 2
Kit Contents and Materials 3
Working RNase Free 4
Preparation of Reagents 5
Starting Materials 5
Protocol for DNA from FFPE samples 6
Protocol for RNA from FFPE samples 8
Troubleshooting Guide 10
Ordering Information 11
Document Revision History 11

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Introduction and Principle

The Clean FFPE DNA & RNA Kit is designed for both DNA and RNA isolation from the same formalin-fixed, paraffin-embedded (FFPE) tissue sample. This process provides the option for a more comprehensive analysis from precious FFPE samples.

The protocol utilizes a proprietary buffer system that not only partially reverses the formaldehyde-induced crosslinking but also ensures DNA and RNA are differentially purified while avoiding cross-contamination.

With our specially formulated lysis buffer, samples are lysed and the DNA is selectively bound to our magnetic CleanNA CFFPE particles. The supernatant can then be removed and processed separately for RNA isolation.

The magnetic particles are separated from solution using a magnetic separation device. With a few rapid wash steps trace contaminants are removed. The purified DNA and/or RNA are then eluted from the CleanNA CFFPE particles using an elution buffer or molecular biology grade water.

The protocol is fully scalable and due to the use of our magnetic bead purification technology can, besides manual usage, easily be automated once the samples have been lysed on liquid handling workstations (Dynamic Devices LYNX™, Hamilton STAR™).

After completing the protocol, the isolated and purified DNA and RNA samples are directly suitable for a variety of downstream applications including SNP analysis, next generation sequencing, genotyping and (q)(RT)PCR.

Kit Contents and Materials

Kit Contents:

Product	CFFPE-DR0096	CFFPE-DR0384	Storage
Preps	96	384	n/a
CFFPE Lysis Buffer	35 mL	140 mL	15-25°C
Proteinase K Solution	2.2 mL	8.8 mL	15-25°C (for storage > 12 months, store at 2-8°C)
CFFPE Binding Buffer	75 mL	250 mL	15-25°C
CleanNA Particles CFFPE	2.2 mL	10 mL	2-8°C
CFFPE Wash Buffer	25 mL	100 mL	15-25°C
Elution Buffer	30 mL	125 mL	15-25°C
DNase Digestion Buffer	25 mL	2 x 25 mL	-20°C
CleanNA DNase I	220 µL	4 x 220 µL	-20°C
CFFPE RNA Rebind Buffer	10 mL	50 mL	-20°C (store at room temperature after addition of ethanol)
Molecular Biology Grade Water	30 mL	125 mL	15-25°C

Note: Cold temperatures during shipment or storage conditions can cause precipitates to form in CFFPE Lysis Buffer. Please check the lysis buffer before starting the isolation procedure for precipitates. Any precipitants can be dissolved by warming the CFFPE Lysis Buffer to 37°C while shaking gently.

Materials and Reagents to be supplied by User:

- 100% ethanol
- 80% ethanol
- 100% isopropanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- 96-well Processing Plates with at least a 2 mL capacity compatible with the magnetic stand used
- Magnetic separation device (CleanNA, Part# CMAG-RN50)
- Sealing film
- Two 96-well plates (for eluted DNA and RNA)
- Incubator capable to be set at 56°C and 90°C
- Centrifuge with swing bucket rotor capable of reaching 2,000g
- Rotor adaptor for 96-well deep-well plates
- Vortexer

Working RNase Free

For RNA applications it is important to work RNase free. RNases are present everywhere and general precautions should be taken to avoid the introduction of RNases and other contaminating nucleases while working with RNA.
The most common sources of RNases are hands, dust particles and contaminated laboratory solutions, equipment and glassware.

To minimize the risk of RNase contamination we recommend the following precautions:

- Always use gloves when handling RNA samples. Change your gloves frequently, to avoid contaminations;
- Ensure to use RNase free filter tips for pipetting;
- Use materials such as disposable consumables, which are guaranteed RNase free;
- Use reagents which are guaranteed RNase free. Creating aliquots from buffers lowers the risk of RNase contamination in buffers, reagents, etc.;
- Avoid using reagents, consumables and equipment dedicated for common use or general lab processes;
- If possible work in a separate room, fume hood or lab space;
- Clean all working surfaces with commercial RNase inhibiting surfactant or 70% ethanol before starting your work / experiment.

Preparation of Reagents

CFFPE RNA Rebind Buffer
Dilute CFFPE RNA Rebind Buffer as follows and store at room temperature.

Kit	100% Ethanol to be Added
CFFPE-DR0096	20 mL
CFFPE-DR0384	100 mL

CFFPE Wash Buffer
Dilute CFFPE Wash Buffer with 100% isopropanol and store at room temperature.

Kit	100% Isopropanol to be Added
CFFPE-DR0096	25 mL
CFFPE-DR0384	100 mL

Starting Materials

Nucleic Acids isolated from FFPE samples are known to be fragmented. A significant percentage of this fragmentation occurs during the paraffin embedding procedures commonly used within laboratories. We recommend the following guidelines to minimize the extent of DNA/RNA fragmentation:

1. Use 4-10% formalin to fix tissue samples
2. Limit the fixation time to 14-24 hours
3. Completely dehydrate samples before embedding
4. Always use freshly cut sections of FFPE tissue

For first time users, we recommend using less than 3 FFPE sections of 10 µm thickness. Depending on the yield and purity obtained, it may be possible to increase the starting material.

Protocol for DNA from FFPE samples

This protocol allows for the isolation of DNA from FFPE sample material. After first particle separation, the supernatant can be transferred and saved for RNA isolation (page 6).

Before Starting:

- Set incubator to 90°C.
- Set incubator to 56°C.
- Prepare CFFPE RNA Rebind Buffer and CFFPE Wash Buffer according to the Preparing Reagents section on Page 4.
- Prepare 80% ethanol.

Protocol:

1. Transfer the FFPE samples to a 96-well Processing Plate with a well capacity of at least 2.0 mL (not provided).
2. Add 300 µL mineral oil to the 96-well Processing Plate.
3. Seal the 96-well Processing Plate with sealing film (not provided).
4. Incubate sample at 56°C for 3 minutes.
5. Remove the sealing film from the 96-well Processing Plate.
6. Add 300 µL CFFPE Lysis Buffer and 20 µL Proteinase K Solution.

Note: CFFPE Lysis Buffer and Proteinase K Solution can be prepared as a mastermix. Prepare only what is needed for each run.

7. Seal the 96-well Processing Plate with sealing film.
8. Centrifuge for 1 minute at 2,000g to create two phases within the solution: an upper oil phase and a lower aqueous phase.

Note: The upper mineral oil layer may turn opaque or cloudy but this will not affect DNA or RNA extraction.

9. Incubate the 96-well Processing Plate at 56°C for 4 hours. If necessary, extend the incubation to overnight or until the tissue is completely lysed.
10. Incubate at 90°C for 1 hour.
11. Centrifuge at 2,000g for 1 minute.
12. Remove the sealing film from the 96-well Processing Plate.
13. Transfer 200 µL of the lower aqueous layer to a new 96-well Processing Plate capable of at least 2.0 mL (not provided). Avoid disturbing the mineral oil layer as much as possible.
14. Add 500 µL CFFPE Binding Buffer and 10 µL CleanNA Particles CFFPE. Vortex samples for 10 minutes.

Note: CFFPE Binding Buffer and CleanNA Particles CFFPE 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.

15. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.

16. Aspirate the supernatant and transfer to a new 96-well Processing Plate with a well capacity of at least 2.0 mL. Seal and store the plate at room temperature. This supernatant can be used for RNA Purification on page 8.

Note: Store the RNA-containing supernatant at room temperature until DNA extraction is completed.

17. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
18. Add 400 µL CFFPE Wash Buffer to the 96-well Processing Plate (containing DNA-bound magnetic particles). Vortex for 2 minutes.

Note: CFFPE Wash Buffer must be diluted with 100% isopropanol prior to use. Please see instructions on Page 4.

19. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
21. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
22. Add 400 µL 80% ethanol (not provided). Vortex for 2 minutes.

Note: Prepare enough 80% ethanol for all wash steps.

23. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles are completely cleared from solution.
24. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
25. Repeat Steps 21-24 for a second 80% ethanol wash step.
26. Leave the 96-well Processing Plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the CleanNA Particles CFFPE for an additional 10 minutes.
27. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
28. Add 50-200 µL Elution Buffer to elute DNA from the CleanNA Particles CFFPE.
29. 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.

30. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles are completely cleared from solution.
31. Transfer the cleared supernatant containing purified DNA to a clean 96-well plate (not provided).
32. Use the purified DNA immediately or store DNA at -20°C. Create appropriate aliquots to avoid repeated freezing/thawing.

Protocol for RNA from FFPE samples

This protocol allows for the isolation and purification of RNA from the first supernatant collected at step 16 during the DNA isolation procedure on page 6.

Before Starting:

- Set incubator to 90°C.
- Set incubator to 56°C.
- Prepare CFFPE RNA Rebind Buffer and CFFPE Wash Buffer according to the Preparing Reagents section on Page 4.
- Prepare 80% ethanol.

Protocol:

1. Start with the supernatant from Step 16 in DNA Purification Protocol. Add 600 µL 100% isopropanol and 10 µL CleanNA Particles CFFPE.

Note: Isopropanol and CleanNA Particles CFFPE can be prepared as a mastermix. Prepare only what is needed for each run.

2. Vortex or tip mix for 10 minutes.

Note: If constant vortexing for 10 minutes is not possible, vortex for 30 seconds every 2 minutes for 10 minutes. Proper mixing is crucial to efficiently bind RNA to CleanNA Particles CFFPE.

3. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
4. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
5. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
6. Add 400 µL 80% ethanol. Vortex for 2 minutes.

Note: Prepare enough 80% ethanol for all wash steps.

7. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
8. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
9. Leave the 96-well Processing Plate on the magnetic separation device for 3 minutes to air dry the CleanNA Particles CFFPE. Remove any residual liquid with a pipettor.
10. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
11. Add 73.5 µL DNase Digestion Buffer and 1.5 µL CleanNA DNase I. Pipet up and down 20 times to mix.

Note: A mastermix of DNase Digestion Buffer and CleanNA DNase I can be made. Prepare only what is needed for each run.

12. Incubate at room temperature for 15 minutes.

13. Add 225 µL CFFPE RNA Rebind Buffer. Vortex for 5 minutes.

Note: CFFPE RNA Rebind Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

Note: If constant vortexing for 5 minutes is not possible, vortex for 30 seconds every 2 minutes for 5 minutes.

14. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
15. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
16. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
17. Add 400 µL 80% ethanol. Vortex for 2 minutes.
18. Place the 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CFFPE.
20. Repeat Steps 16-19 for a second 80% ethanol wash step.
21. Leave the 96-well Processing Plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the CleanNA Particles CFFPE for an additional 10 minutes.
22. Remove the 96-well Processing Plate containing the CleanNA Particles CFFPE from the magnetic separation device.
23. Add 50-200 µL molecular biology grade water to elute RNA from the CleanNA Particles CFFPE.
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 96-well Processing Plate on the magnetic separation device to magnetize the CleanNA Particles CFFPE. Incubate at room temperature until the CleanNA Particles CFFPE are completely cleared from solution.
26. Transfer the cleared supernatant containing purified RNA to a clean 96-well plate (not provided).
27. Use the purified RNA immediately or store RNA at -80°C. Create appropriate aliquots to avoid repeated freezing/thawing.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yield	Sample was not completely lysed after 4 hours.	Let the lysis proceed overnight at 56°C.
	Incomplete resuspension of magnetic particles.	Resuspend the magnetic particles by vortexing before use.
	CFFPE Wash Buffer was not prepared correctly.	Prepare CFFPE Wash Buffer according to the instructions on Page 4.
	Loss of magnetic particles during operation.	Increase the particle collection/ magnetization time.
Low RNA Yields	CFFPE RNA Rebind Buffer was not prepared correctly.	Prepare CFFPE RNA Rebind Buffer according to the instructions on Page 4.
	Incomplete resuspension of magnetic particles.	Resuspend the magnetic particles by vortexing before use.
	Loss of magnetic particles during operation.	Increase the particle collection/ magnetization time.
Carryover of the magnetic particles in the elution	Carryover of the magnetic particles in the eluted DNA or RNA will not affect downstream applications.	To remove the carryover magnetic particles from the eluted DNA or RNA, simply magnetize the magnetic particles and carefully transfer the DNA or RNA eluate to a new tube or plate.
DNA contamination	Incomplete digestion of DNA during DNase Digestion Step.	After Step 10 of the RNA Purification Protocol, perform the following steps: • Add 100 µL molecular biology grade water to each sample and vortex for 5 minutes. • Add 73.5 µL DNase Digestion Buffer and 1.5 µL CleanNA DNase I. • Incubate at room temperature for 15 minutes. • Add 525 µL CFFPE RNA Rebind Buffer and vortex for 5 minutes. • Continue with Step 14 of the RNA Purification Protocol. This will require additional CFFPE RNA Rebind Buffer that is not provided with this kit. Contact your local distributor for more information.
Problem with downstream applications	DNA is excessively cross-linked due to over- fixation.	Extend incubation time at 90°C (step 10 on page 6) to 90 minutes.

Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean FFPE DNA & RNA Kit (96 Preps)	CFFPE-DR0096
Clean FFPE DNA & RNA Kit (384 Preps)	CFFPE-DR0384

Product	Part Number
Clean Magnet Plate 96-Well	CMAG-96-RN50

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
5.00	October 2021	Total revision.	Language and layout modifications.
4.00	September 2020	Protocol for RNA.	Improved Title.
3.00	September 2020	Total revision.	New layout.
			Important general information added at page 1 (before contents).

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