omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® FFPE DNA/RNA 96 Kit

M6955-00	1 x 96 preps
M6955-01	4 x 96 preps

Manual Date: January 2024
Manual Revision: v3.3

For Research Use Only

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Mag-Bind® FFPE DNA/RNA 96 Kit

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Introduction

Mag-Bind® FFPE DNA/RNA 96 Kit is designed for the sequential isolation of DNA and RNA in separate eluates from the same formalin-fixed, paraffin-embedded (FFPE) tissue sample. The purification of DNA and RNA from the same sample enables a more comprehensive analysis from a precious sample source. The protocol utilizes a specially formulated buffer system that not only partially reverses the formaldehyde-induced crosslinking but also ensures DNA and RNA are differentially purified with no cross-contamination. The kit can also be used for purification of only DNA or only RNA from FFPE samples if sequential isolation is not desired. Magnetic bead-based extraction makes it suitable for both manual as well as automated processing. Purified DNA and RNA are suitable for a variety of downstream applications including SNP analysis, next generation sequencing, and genotyping.

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 informati

New In This Edition:

January 2024:
- Addition of Warnings and Safety Information.

May 2022:
- An important statement is included clarifying how the actual number of preparations is dependent on various factors and may be lower than the number of preparations specified with the kit.

January 2020:
- A new protocol for the extraction of RNA only from FFPE has been added to the kit.

Principle

The Mag-Bind® FFPE DNA/RNA 96 Kit integrates a unique buffer system with the highly efficient binding properties of Mag-Bind® technology to isolate total DNA and RNA in separate eluates from the same FFPE sample. The protocol utilizes non-toxic mineral oil in combination with heat for efficient deparaffinization of the FFPE sample eliminating the use of hazardous xylene.

Samples are first lysed in FDR Buffer aided by the presence of Proteinase K enzyme. The lysate is then heated to denature the proteinase and reverse the chemical crosslinking of the nucleic acids. Post-heating, the lysate is mixed with MB4 Buffer and Mag-Bind® Particles CH to bind DNA to the particles. The RNA-containing supernatant is saved and a second binding step is completed with addition of isopropanol to bind RNA to the Mag-Bind® Particles CH. This results in separation of DNA and RNA into two fractions. Mag-Bind® Particles bound to DNA and RNA are individually washed and nucleic acids are eluted in two different tubes for further analyses. Only DNA or RNA can be isolated following the appropriate protocols outlined in the manual.

Starting Materials

Standard formalin-fixation, paraffin embedding procedures cause significant fragmentation of nucleic acids. We recommend the following guidelines to limit 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. 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.

Kit Contents

Product	M6955-00	M6955-01
Purifications	1 x 96	4 x 96
FDR Buffer	35 mL	140 mL
MB4 Buffer	75 mL	250 mL
RMP Buffer	25 mL	100 mL
Elution Buffer	30 mL	125 mL
Nuclease-free Water	30 mL	125 mL
DNase Digestion Buffer	25 mL	2 x 25 mL
PHM Buffer	10 mL	50 mL
Proteinase K Solution	2.2 mL	8.8 mL
Mag-Bind® DNase I	220 µL	4 x 220 µL
Mag-Bind® Particles CH	2.2 mL	10 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® FFPE DNA & RNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® DNase I, DNase Digestion Buffer, and PHM Buffer should be stored at -20°C. Store PHM Buffer at room temperature after addition of ethanol. 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. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

Please take a few minutes to read this manual thoroughly to become familar with the protocol before beginning the procedure. To minimize RNA degradation, prepare all required materials before starting. Wear gloves/protective goggles and take great care when working with chemicals.

1. Dilute PHM Buffer with 100% ethanol and store at room temperature.

Kit	100% Ethanol to be Added
M6955-00	20 mL
M6955-01	100 mL

2. Dilute RMP Buffer with 100% isopropanol and store at room temperature.

Kit	100% Isopropanol to be Added
M6955-00	25 mL
M6955-01	100 mL

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

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.

Some of the buffers included in the product 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 waste. Please access the SDSs online for detailed information on the reagents.

Sequential Protocol

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

Note: For DNA purification only, please follow this procotol until the end of Step 32 of the DNA Purification Procedure.

Materials and Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 56°C and 90°C
- 96-well processing plates with at least a 2 mL capacity compatible with the magnetic separation device used
- 96-well microplate (for eluted RNA)
- 80% ethanol
- 100% ethanol
- 100% isopropanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before Starting:

- Prepare PHM Buffer and RMP Buffer according to the Preparing Reagents section on Page 5.
- Prepare 80% ethanol.
- Set incubator to 56°C.
- Set incubator to 90°C.

DNA Purification Procedure

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 (not provided) to the 96-well processing plate.

3. Seal the 96-well processing plate with sealing film (not provided).

4. Incubate at 56°C for 3 minutes.

5. Remove the sealing film from the 96-well processing plate.

6. Add 300 µL FDR Buffer and 20 µL Proteinase K Solution.

   Note: FDR 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 at 2,000*g* for 1 minute 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 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 2,000*g* 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. Avoid disturbing the mineral oil layer as much as possible.

14. Add 500 µL MB4 Buffer and 10 µL Mag-Bind® Particles CH. Vortex for 10 minutes.

    Note: MB4 Buffer and Mag-Bind® Particles CH 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 Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH 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. The supernatant will be used for RNA Purification on Page 11!**

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

    Optional: If only DNA is desired, do not save the supernatant for RNA extraction.

17. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

18. Add 400 µL RMP Buffer to the 96-well processing plate (containing DNA-bound magnetic beads). Vortex for 2 minutes.

    Note: RMP Buffer must be diluted with 100% isopropanol prior to use. Please see instructions on Page 5.

19. Place the 96-well processing 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.

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

21. Remove the 96-well processing plate containing the Mag-Bind® Particles CH 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 Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles are completely cleared from solution.

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

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 Mag-Bind® Particles CH for an additional 10 minutes.

27. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

28. Add 50-200 µL Elution Buffer to elute DNA from the Mag-Bind® Particles CH.

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 Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles are completely cleared from solution.

31. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided).

32. Store DNA at -20°C.

    Optional: If only DNA is desired, skip the RNA Purification Procedure.

RNA Purification Procedure

1. Start with the supernatant from Step 16 in DNA Purification Protocol. Add 600 µL 100% isopropanol and 10 µL Mag-Bind® Particles CH.

   Note: Isopropanol and Mag-Bind® Particles CH 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 Mag-Bind® Particles CH.

3. Place the 96-well processing 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.

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

5. Remove the 96-well processing plate containing the Mag-Bind® Particles CH 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 Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

9. Leave the 96-well processing plate on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette.

10. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

11. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind® DNase I. Pipet up and down 20 times to mix.

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

12. Let sit at room temperature for 15 minutes.

13. Add 225 µL PHM Buffer. Vortex for 5 minutes.

    Note:
    1. PHM Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

    2. 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 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. Remove the 96-well processing plate containing the Mag-Bind® Particles CH 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 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.

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 Mag-Bind® Particles CH for an additional 10 minutes.

22. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

23. Add 50-200 µL Nuclease-free Water.

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 Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

26. Transfer the cleared supernatant containing purified RNA to a clean 96-well microplate (not provided).

27. Store RNA at -80°C.

RNA Only Protocol

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 Equipment to be Supplied by User:

- Centrifuge with swing bucket rotor capable of 2,000*g*
- Rotor adaptor for 96-well deep-well plates
- Magnetic separation device
- Vortexer
- Incubator capable of 56°C and 90°C
- 96-well processing plates with at least a 2 mL capacity compatible with the magnetic separation device used
- 96-well microplate (for eluted RNA)
- 80% ethanol
- 100% ethanol
- 100% isopropanol
- Mineral oil (Recommend VWR, Cat# 97064-128)
- Sealing film (Recommend Omega Bio-tek, Cat# AC1200)

Before Starting:

- Prepare PHM Buffer according to the Preparing Reagents section on Page 5.
- Prepare 80% ethanol.
- Set incubator to 56°C.
- Set incubator to 90°C.

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 (not provided) to the 96-well processing plate.

3. Seal the 96-well processing plate with sealing film (not provided).

4. Incubate at 56°C for 3 minutes.

5. Remove the sealing film from the 96-well processing plate.

6. Add 300 µL FDR Buffer and 20 µL Proteinase K Solution.

   Note: FDR 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 at 2,000*g* for 1 minute 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 RNA extraction.

9. Incubate at 56°C for 90 minutes. 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 2,000*g* 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. Avoid disturbing the mineral oil layer as much as possible.

14. Add 500 µL MB4 Buffer, 10 µL Mag-Bind® Particles CH, and 600 µL 100% isopropanol. Vortex or tip mix samples for 10 minutes.

    Note:
    1. MB4 Buffer and Mag-Bind® Particles CH can be prepared as a mastermix. Prepare only what is needed for each run.

    2. 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 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 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

18. Add 400 µL 80% ethanol (not provided). Vortex for 2 minutes.

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

19. Place the 96-well processing 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.

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

21. Leave the 96-well processing plate on the magnetic separation device for 3 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipette.

22. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

23. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind® DNase I. Pipet up and down 20 times to mix.

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

24. Let sit at room temperature for 15 minutes.

25. Add 225 µL PHM Buffer. Vortex for 5 minutes.

    Note:
    1. PHM Buffer must be diluted with 100% ethanol prior to use. Please see instructions on Page 5.

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

26. Place the 96-well processing 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.

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

28. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

29. Add 400 µL 80% ethanol. Vortex for 2 minutes.

30. Place the 96-well processing 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.

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

32. Repeat Steps 28-31 for a second 80% ethanol wash step.

33. Leave the 96-well processing plate on the magnetic separation device. Wait 1 minute. Remove residual liquid with a pipette. Air dry the Mag-Bind® Particles CH for an additional 10 minutes.

34. Remove the 96-well processing plate containing the Mag-Bind® Particles CH from the magnetic separation device.

35. Add 50-200 µL Nuclease-free Water.

36. 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.

37. Place the 96-well processing 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.

38. Transfer the cleared supernatant containing purified RNA to a clean 96-well plate (not provided).

39. Store RNA at -80°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 Yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	RMP Buffer was not prepared correctly	Prepare RMP Buffer according to the instructions on Page 5.
	Loss of magnetic particles during operation	Increase the particle collection/ magnetization time.
	Sample was not completely lysed after 4 hours	Let the lysis proceed overnight at 56°C.
Problem	**Cause**	**Solution**
Low RNA Yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	PHM Buffer was not prepared correctly	Prepare PHM Buffer according to the instructions on Page 5.
	Loss of magnetic particles during operation	Increase the particle collection/ magnetization time.
Problem	**Cause**	**Solution**
Problem with downstream application	DNA is excessively cross-linked due to over-fixation	Extend incubation time at 90°C to 90 minutes.
Problem	**Cause**	**Solution**
Carryover of the magnetic particles in the elution	Carryover of the magnetic particles in the eluted DNA or RNA will not effect 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.

Problem	Cause	Solution
DNA contamination	Incomplete digestion of DNA during DNase Digestion Step	After Step 10 of the RNA Purification Protocol (Page 12) or after Step 22 of RNA Only Protocol (Page 16), perform the following: 1. Add 100 µL Nuclease-free Water to each sample and vortex for 5 minutes. 2. Add 73.5 µL DNase Digestion Buffer and 1.5 µL Mag-Bind DNase I. 3. Let sit at room temperature for 15 minutes. 4. Add 525 µL PHM Buffer and vortex for 5 minutes. 5. Continue with Step 14 of the RNA Purification Protocol (Page 12) or Step 26 of the RNA only Protocol (Page 17). This will require additional PHM Buffer that is not provided with this kit. Please contact your Omega Bio-tek representative at **1-800-832-8896** for ordering information.

Notices & Disclaimers

For European Union Use.

MB4 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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For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


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Plasmid


Cultured Cells


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NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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