FFPE Protein Extraction Solution

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Catalog #400926

Protocol

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Research Use Only. Not for use in Diagnostic Procedures.

Agilent Technologies

Notices

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Agilent Technologies, Inc.
5301 Stevens Creek Rd
Santa Clara, CA 95051 USA

Technical Support

For technical product support, contact Agilent at (800) 894-1304 or techservices@agilent.com.

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In this Guide...

This document describes how to remove paraffin from FFPE tissue, then to use FFPE Protein Extraction Solution to extract protein from FFPE tissue.

If you have comments about this protocol, send an e-mail to proteomics.support@agilent.com.

1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

2 Procedures

This chapter contains information on how to remove paraffin and extract protein from FFPE tissue. It also contains troubleshooting information.

Contents

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1 Before You Begin

Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

Procedural Notes

- The yield of protein obtained with the FFPE protein extraction solution may vary between samples due to differences in the way the tissue samples were fixed and embedded and how well the proteins have been preserved. When the quality of protein preservation in the FFPE sample is in question, increase the amount of starting material used in the protocol.
- When sections are cut from a block of tumor-derived tissue, the samples will often contain a mix of tumor cells and adjacent normal cells. In selecting which samples to work with for your downstream analyses, take into consideration the heterogeneity that may exist between and within the tissue sections.

Kit contents

Table 1 Kit Contents

Product Name	Catalog #400925*	Catalog #400926†
Protein Extraction Solution	1.25 mL	4x1.25 mL

* Enough solution for 25 protein extractions.

† Enough solution for 100 protein extractions.

Conditions

Store the FFPE Protein Extraction Solution at -80°C upon receipt. Once thawed, divide the solution into single-use aliquots. Aliquots can be stored at -20°C for 6 months, or at -80°C for long term storage.

Required equipment and supplies

Table 2 Required Equipment

Description
Xylene
100% Ethanol
85% Ethanol
70% Ethanol
Protease-free dH_20
Microcentrifuge tubes

Overview

Formalin fixation followed by paraffin-embedding of tissue sections is a common histology technique that allows the tissue samples to be preserved for years. With the development of new proteomics technologies, there is an increasing demand for the ability to extract proteins from formalin-fixed paraffin-embedded (FFPE) tissues without the use of detergents that can permanently denature protein epitopes and interfere with subsequent downstream analyses.

The Agilent FFPE Protein Extraction Solution is a detergent-free reagent for extraction of full-length proteins and short polypeptides from FFPE tissues. The solution is designed to preserve protein epitopes to facilitate antibody recognition. Following extraction, samples may be utilized for downstream applications such as ELISA, immunoprecipitation, SDS-PAGE, and western blot assays. In addition, because the extraction solution is detergent-free, samples can be directly analyzed via LC/MS to identify protein biomarkers or profile protein pathways.

2 Procedures

This chapter contains information on how to remove paraffin and extract protein from FFPE tissue. It also contains troubleshooting information.

To remove paraffin from tissue sections cut from a block

1. From a block of FFPE tissue, cut sections up to 10 µm thick and with an area of 40 to 100 mm^2.
2. Immediately transfer the sections to sterile 1.5-mL microcentrifuge tubes. Up to 2 sections may be placed in the same tube.
3. Wash the FFPE tissue in xylene 3 times:
   - **a** Use a pipet to add 1 mL of xylene into each tube and mix on a vortex mixer for 10 seconds.
   - **b** Incubate the tubes at room temperature for 10 minutes.
   - **c** Spin the tubes in a microcentrifuge for 2 minutes at full speed. The tissue will collect at the bottom of the tube forming a loose pellet.
   - **d** Without disturbing the pellet, carefully remove and discard the supernatant using a pipet.
   - **e** Repeat entire step for a total of 3 xylene washes.
4. Wash the tissue in 100% ethanol 2 times:
   - **a** Use a pipet to add 1 mL of 100% ethanol into each tube and mix on a vortex mixer.
   - **b** Incubate the tubes at room temperature for 5 minutes.
   - **c** Spin the tubes in a microcentrifuge for 2 minutes at full speed.
   - **d** Without disturbing the pellet, use a pipet to carefully remove and discard the supernatant.
   - **e** Repeat entire step for a total of 2 ethanol washes.
5. Wash the tissue in 85% ethanol 2 times:
   - **a** Use a pipet to add 1 mL of 85% ethanol into each tube and mix on a vortex mixer.
   - **b** Incubate the tubes at room temperature for 1 minute.
   - **c** Spin the tubes in a microcentrifuge for 2 minutes at full speed.
   - **d** Without disturbing the pellet, use a pipet to carefully remove and discard the supernatant.
   - **e** Repeat entire step for a total of 2 ethanol washes.

6 Wash the sample in 70% ethanol 2 times:

a Use a pipet to add 1 mL of 70% ethanol into each tube and mix on a vortex mixer.

b Incubate the tubes at room temperature for 1 minute.

c Spin the tubes in a microcentrifuge for 2 minutes at full speed.

d Without disturbing the pellet, use a pipet to carefully remove and discard the supernatant.

If the pellet becomes dislodged while removing the supernatant, spin the tube again.

e Repeat entire step for a total of 2 ethanol washes.

7 Continue at "To extract protein" on page 15.

See Table 3 for a summary of the steps required to remove paraffin from FFPE tissues.

Table 3 Summary of steps to remove paraffin. Carefully remove and discard supernatant between steps.

Repetition	Wash solution (1 mL)	Incubate at room temperature	Spin in microcentrifuge
3 times	Xylene	10 minutes	2 minutes
2 times	100% ethanol	5 minutes	2 minutes
2 times	85% ethanol	1 minute	2 minutes
2 times	75% ethanol	1 minute	2 minutes

To remove paraffin from FFPE tissue sections mounted on a slide

1. Incubate the slide in a horizontal position at room temperature for 1 hour or at 60°C for 20 minutes.
2. Immerse the slide into a staining dish containing xylene. Incubate for 10 minutes at room temperature.

   Each time you are asked to incubate the slide in a liquid solution, make sure the slide is completely submerged.
3. Exchange the xylene in the staining dish for fresh xylene and incubate the slide for another 10 minutes.
4. Immerse the slide into a staining dish containing 100% ethanol. Incubate for 5 minutes at room temperature.
5. Immerse the slide into a dish of 85% ethanol and incubate for 1 minute.
6. Immerse the slide into a dish of 70% ethanol and incubate for 1 minute.
7. Immerse the slide into a dish of protease-free dH_20 and incubate for 1 minute.
8. Remove the slide from the dH_20. Without disturbing the tissue section, use a paper towel to gently wipe off excess liquid from the slide. Do not allow the tissue section to dry out.
9. Use a clean, sterile razor blade to carefully transfer the tissue section from the slide into a 1.5-mL microcentrifuge tube.
10. Continue at "To extract protein" on page 15.

See Table 4 for a summary of the wash steps.

Table 4 Summary of steps to remove paraffin.

Repetition	Wash solution (1 mL)	Incubate at room temperature
2 times	Xylene	10 minutes
1 time	100% ethanol	5 minutes
1 time	85% ethanol	1 minute
1 time	75% ethanol	1 minute
1 time	Protease-free dH_20	1 minute

To extract protein

1. Add 50 µL of the FFPE Protein Extraction Solution to each tube of deparaffinized tissue. Spin on a vortex mixer for 5 seconds.
2. Incubate the tubes at 100°C for 20 minutes.
3. Spin the tubes in a microcentrifuge for 1 minute at 1000×g to bring all the contents to the bottom.
4. Mix on a vortex mixer for 5 seconds.
5. Incubate the tubes at 60°C for 2 hours. Every 30 minutes:
   - Remove the tubes from the incubator and mix well on a vortex mixer for several seconds.
   - Briefly spin the tubes in a centrifuge to remove any condensation and return them to 60°C.
6. Spin the tubes at 4°C in a microcentrifuge for 10 minutes at 15,000×g to collect the cellular debris into a pellet at the bottom of the tube.
7. Transfer the supernatant to a fresh 1.5-mL tube.

   The protein extracts are now ready for downstream applications such as SDS-PAGE, Western blot analyses, ELISA assays and LC/MS analyses. The protein yield may be quantitated using a Bradford assay.

Store the protein extracts at -20°C. The extract will not freeze at -20°C.

If you get low protein yield

- ✔ Increase the amount of starting material.

  The quality of the starting material may not be optimal, or the FFPE tissues have been stored long-term which leads to low extraction efficiency.

- ✔ Some tissue sections mounted on slides may be very loose. Check that you do not dislodge the tissue during the deparaffinization steps.

- ✔ Paraffin may not be completely removed. Repeat the xylene treatment 1 to 2 more times.

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In This Book

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