PRODUCT INFORMATION & MANUAL

FFPE Tissue Extraction Kit

NBP2-37854

For isolation and cleanup of proteins from formalin-fixed, paraffin-embedded tissue sections for western blot, reverse-phase array analyses, mass spectrometry, and 2D-PAGE analysis

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Novus kits are guaranteed for 6 months from date of receipt

Contents

Kit Contents 3

Storage 3

Product Use Limitations 3

Product Warranty and Satisfaction Guarantee 4

Technical Assistance 4

Quality Control 4

Safety Information 5

Introduction 6

Principle and procedure 6

Important Notes 11

Protocols

- **Deparaffinization of FFPE Tissue Sections Mounted on a Microscope Slide** 13
- **Deparaffinization of FFPE Tissue Sections Cut Directly from an FFPE Sample Block** 14
- **Extraction of Total Protein from FFPE Tissue Sections for Western Blot and RPA** 16
- **Extraction of Proteins from FFPE Tissues and Cleanup for 2D-PAGE or MS Analysis** 18

Troubleshooting Guide 23

Appendix: Buffer Preparation 24

Ordering Information 25

Kit Contents

FFPE Tissue Extraction Kit	(20)
Catalog no.	NBP2-37854
Number of preps	20
Extraction Buffer EXB Plus	2 x 1 ml
Collection Tubes (1.5 ml)	50
Collection Tube Sealing	20
Clips Handbook	1

Storage

Extraction Buffer EXB Plus should be stored at –20°C upon arrival. The other components of the FFPE Tissue Extraction Kit should be stored dry at room temperature (15–25°C). Store Heptane, Methanol, and Chloroform from the FFPE Solvent Set in an appropriate solvent cabinet. Under these conditions, the kits are stable for at least 12 months.

Product Use Limitations

FFPE Tissue Extraction Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of Novus products to adhere to the NIH

guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

Novus guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Novus will replace it free of charge or refund the purchase price. Novus reserves the right to change, alter, or modify any product to enhance its performance and design. If a Novus product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

If you have questions about product specifications or performance, please call Novus Technical Services or visit www.novusbio.com).

Technical Assistance

At Novus, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of Novus products. If you have any questions or experience any difficulties regarding the FFPE Tissue Extraction Kits or Novus products in general, please do not hesitate to contact us.

Novus customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at Novus. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.novusbio.com/Support or call the Novus Technical Service Department.

Quality Control

In accordance with QIAGEN’s ISO-certified Quality Management System, each lot of FFPE Tissue Extraction Kit is tested against predetermined specifications to ensure consistent product quality.

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). These are available online in convenient and compact PDF formats.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

A number of proteomic studies have been carried out to elucidate differential protein expression patterns in normal and diseased (e.g., tumor) cells in vitro. The identification of differentially expressed proteins may designate specific tumor markers and could help in monitoring cancer progression or classification of tumor types, resulting in better diagnoses and improved therapies. However, results obtained from cell cultures may not represent the true in vivo expression pattern. Clinical tissue samples represent a comprehensive source of protein expression profiles associated with diseases such as cancer. These samples enable the course of disease — e.g., before and after therapy — to be examined, a process that cannot be easily studied in model systems such as cell cultures.

In tissue specimens used for pathological diagnosis, the standard histopathology and immunohistochemistry fixative formalin is usually used to preserve morphological details. Without further processing, the consequent crosslinking of the proteins in the sample means that this material is unsuitable for proteomic studies.

FFPE Tissue Extraction Kits are used for extracting proteins from formalin-fixed, paraffin-embedded (FFPE) tissue. Extraction efficiency is comparable to that seen from frozen tissue. The new and improved Extraction Buffer EXB Plus ensures higher extraction efficiency and enables extraction of full-length, intact proteins from FFPE blocks that were stored for longer periods or from FFPE tissues which had been over-fixed with formalin. Proteins extracted using the FFPE Tissue Extraction Kit are suitable for western blot or reverse-phase arrays (RPA) and after subsequent sample clean up, are suitable for 2D-PAGE and mass spectrometry (MS) analysis.

Principle and procedure

The FFPE Tissue Extraction Kit procedure provides optimized conditions for extracting total protein from FFPE tissue notably from older FFPE blocks or from formalin over-fixed tissue. After deparaffinization, tissue is incubated in an optimized lysis buffer at 2 different temperatures in a process that reverses formalin cross-linking and untangles protein molecules. After a centrifugation step, the supernatant containing the released proteins is recovered. The extracted proteins can be used for western blot or reverse-phase array analysis. In the next step (optional), the proteins are desalted, removing buffer substances and rendering the sample suitable for 2D-PAGE or MS analysis.

Extraction and Analysis of Proteins from FFPE Tissues

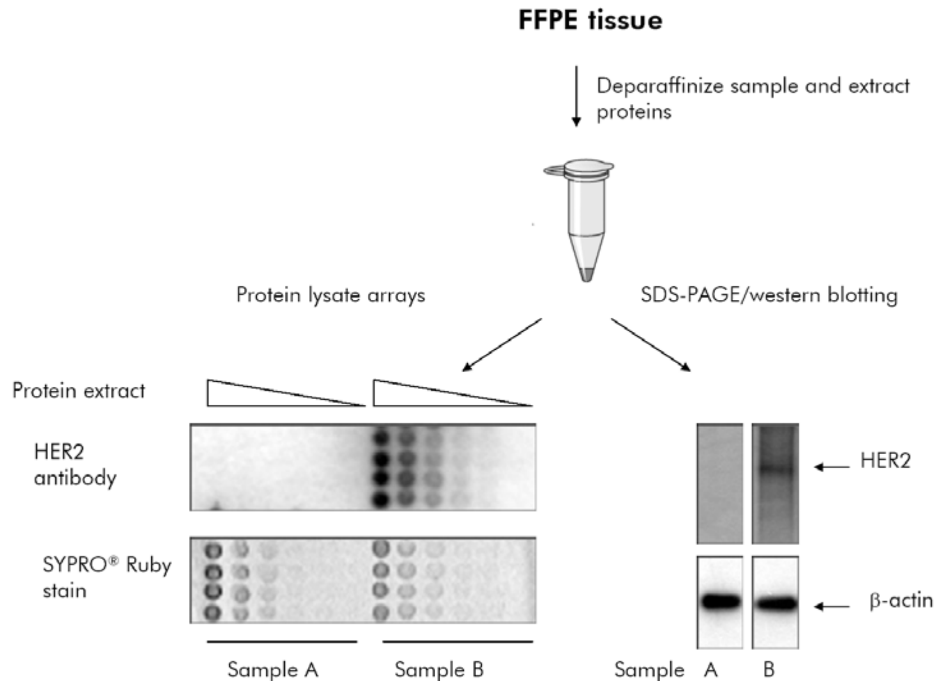


Figure 1. Protein extraction enables comparison of histological samples on the protein level. Two breast cancer biopsy samples, both scored 2+ for the receptor tyrosine kinase HER2 by immunohistochemistry, were processed using the FFPE Tissue Extraction Kit and analyzed by protein lysate array and western blotting. In sample B, HER2 gene amplification was confirmed by FISH (data not shown). The housekeeping gene β-actin was analyzed as a control. Data kindly provided by Karl-Friedrich Becker and Christina Schott, Technical University of Munich, Germany.

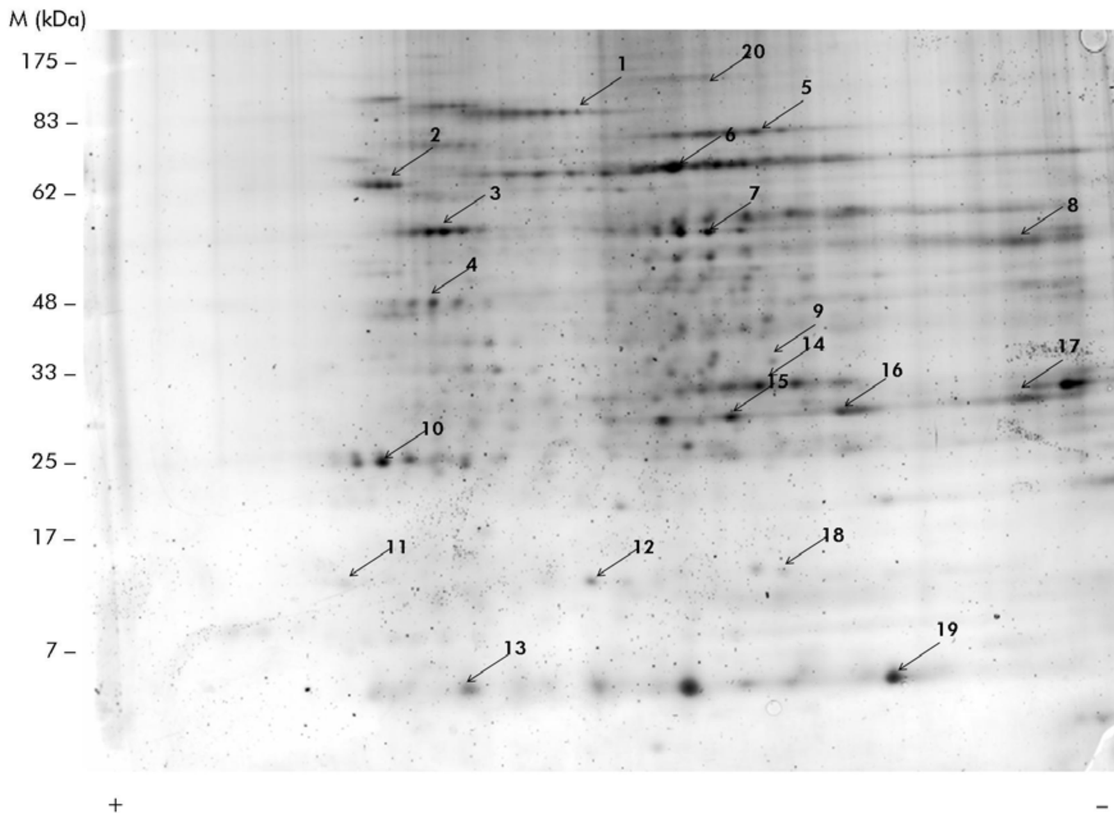


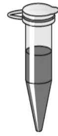
Figure 2. Efficient extraction and identification of proteins from FFPE tissue after 2D-PAGE. Serial sections from rat liver FFPE tissue blocks were prepared using the FFPE Tissue Extraction Kit. Extracted proteins were cleaned up prior to 2D-PAGE analysis (150 µg each) according the protocol on page 19. Protein spots were visualized by SYPRO® staining and the gel was scanned using an Ettan™ DIGE Imager (GE Healthcare). For MALDI-MS analysis of single protein spots, the SYPRO stained gel was destained and stained with Coomassie® Blue. Random spots from different areas were excised and analyzed by MALDI-MS (see Table 1).

Table 1. Protein identifications from 2D-PAGE of FFPE rat liver samples

Spot	IPI entry	Mascot score	Protein	MW (kDa)	pI
1	IPI00191737	477	Alb Serum albumin precursor	70.6	6.09
2	IPI00551812	406	Atp5b ATP synthase subunit beta, mitochondrial precursor	56.3	5.19
3	IPI00765011	474	LOC295810 similar to Actin, cytoplasmic 2	59.1	5.67
4	IPI00389611	196	Rgn Regucalcin	34.0	5.27
5	IPI00231742	417	Cat Catalase	60.0	7.07
6	IPI00396910	136	Atp5a1 ATP synthase subunit alpha, mitochondrial precursor	59.8	9.22
7	IPI00214480	217	Fah Fumarylacetoacetase	46.2	6.67
8	IPI00201413	499	Acaa2 3-ketoacyl-CoA thiolase, mitochondrial	42.2	8.09
9	IPI00205332	460	Etfa Electron transfer flavoprotein subunit alpha, mitochondrial precursor	35.2	8.62
10	IPI00760117	273	Comt Isoform 2 of Catechol O-methyltransferase	25.0	5.11
11	IPI00231013	439	Cyb5 Isoform Short of Cytochrome b5	11.4	5.26
12	IPI00231643	587	Sod1 Superoxide dismutase	16.0	5.88
13	IPI00231292	241	Hrsp12 Ribonuclease UK114	14.5	6.21
14	IPI00230788	477	Ca3 Carbonic anhydrase 3	29.7	6.89
15	IPI00411230	298	Gstm2 Glutathione S-transferase Mu 2	25.8	6.90
16	IPI00411230	540	Gstm2 Glutathione S-transferase Mu 2	25.8	6.90
17	IPI00231639	494	Gstm1 Glutathione S-transferase Mu 1	26.0	8.27
18	IPI00325189	372	Nme2 Nucleoside diphosphate kinase B	17.3	6.92
19	IPI00190790	433	Fabp1 Fatty acid-binding protein, liver	14.3	7.79
20	IPI00679202	150	Tf Isoform 1 of Serotransferrin precursor	78.5	7.14

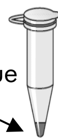
FFPE Protein Isolation Procedure

FFPE tissue sections

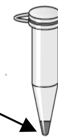


Use Xylene for deparaffinization if the downstream application is western blot or RPA. Use Heptan for deparaffinization if the downstream application is 2D-PAGE or MS analysis.

Add 100 µl Extraction Buffer EXB Plus followed by heat treatment

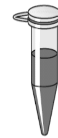


Transfer supernatant into a new tube after centrifugation



Extracted proteins for western blot or RPA

Add methanol, chloroform, and water



Remove organic phase and pellet protein by adding methanol

Resuspend protein pellet in 2D-PAGE sample or MS lysis buffer

Important Notes

Starting material

Use standard formalin-fixation and paraffin-embedding procedures for the preparation of FFPE tissue sections. To optimize protein recovery, ensure the following criteria are met during fixation and embedding:

- Fix tissue samples in 4–10% formalin as quickly as possible after surgical removal
- Use a fixation time of 14–24 hours (longer fixation times will result in poor protein extraction efficiency)
- Thoroughly dehydrate samples prior to paraffin embedding
- Suitable starting materials are FFPE tissue sections cut directly from an FFPE sample block or unstained FFPE sections mounted on a microscope slide (e.g., sections from a series of FFPE tissue sections that could be used for histological or immunohistological analysis but have not been stained, for example with hematoxylin/eosin).

Deparaffinization

Up to 3 sections, each with a thickness of up to 15 μm and an area of up to 100 mm^2, can be combined in one preparation. It is also possible to use smaller sections ($\geq$25 mm^2) for one preparation. The yield of extracted protein depends on the amount and the nature of the starting material and may vary. Table 2 gives an overview of protein yields from different tissues and amounts of starting material. If you are not sure how much protein your sample contains, we recommend using 2 sections, each with a thickness of 10–15 μm and an area of 100 mm^2 per preparation.

Table 2. Protein yields from different starting materials

Tissue	No. of sections	Total size (mm^2)	Protein yield
A431 xenograft (mouse)*	3	~1000	388 µg
H1975 xenograft (mouse)*	3	~1200	301 µg
Colon normal (human)*†	5	~2500	530 µg
Barrett carcinoma (human)*†	5	~2500	470 µg
Lymph node normal (human)*†	5	~2500	258 µg
Pancreas normal (human)*†	5	~2500	862 µg
Gastric cancer (human)*†	5	~2500	638 µg
Kidney (rat)*	3	~500–750	328 µg
Brain (rat)*	3	~150–200	148 µg

* Proteins were extracted from FFPE tissue sections (10 µm) cut directly from a FFPE tissue sample block.

† Data kindly provided by Karl-Friedrich Becker and Christina Schott, Institute of Pathology, Technical University of Munich, Germany.

Protocol: Deparaffinization of FFPE Tissue Sections Mounted on a Microscope Slide

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, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Xylene
- 100%, 96%, and 70% (v/v) ethanol*
- Container/tank for deparaffinization of FFPE section
- Needles

Important points before starting

- If using the FFPE Tissue Extraction Kit the first time, read the “Important Notes” section on page 12.
- Xylene washes (steps 1 and 2) should be performed in a fume hood.

Procedure

1. **Transfer the slide to a suitable reservoir containing xylene. The slide should be completely covered with xylene. Incubate for 10 min at room temperature (15–25°C).**
2. **Repeat step 1 twice, using fresh xylene each time.**
3. **Incubate the slide in 100% ethanol* for 10 min at room temperature (15–25°C). Repeat this step using fresh 100% ethanol.**
4. **Transfer to a reservoir containing 96% ethanol* and incubate for 10 min. Repeat this step using fresh 96% ethanol.**
5. **Transfer to a reservoir containing 70% ethanol* and incubate for 10 min. Repeat this step using fresh 70% ethanol.**
6. **Immerse the slide in double-distilled water for 30 s and remove water by tapping the slide carefully on a paper towel.**

   Note: Do not touch the section with the paper towel. Ensure that the sections do not dry out.
7. **Excise areas of interest with a needle (not supplied) and transfer to a 1.5 ml collection tube (supplied).**
8. **Proceed immediately with the protocol “Extraction of Total Protein from FFPE Tissue Sections for Western Blot and RPA” on page 17.**

* Do not use denatured alcohol that contains other substances such as methanol or methylethylketone.

Protocol: Deparaffinization of FFPE Tissue Sections Cut Directly from an FFPE Sample Block

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, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Microcentrifuge (with rotor for 1.5 ml tubes)
- Vortexer
- Xylene
- 100%, 96%, and 70% (v/v) ethanol*

Important points before starting

- If using the FFPE Tissue Extraction Kit the first time, read the “Important Notes” section on page 12.
- All centrifugation steps are carried out at 20–25°C using a bench-top microcentrifuge (e.g., Eppendorf® Micro Centrifuge 5417C or Heraeus Biofuge® 15).
- Xylene washes should be performed in a fume hood.

Procedure

1. **Cut up to 3 serial 10–15 *µ*m thick sections from the same block.**
2. **Immediately place the sections in a 1.5 ml collection tube (supplied).**
3. **Pipet 1 ml xylene into the tube. Vortex vigorously for 10 s and incubate for 10 min.**
4. **Centrifuge the tube in a microcentrifuge at full speed for 2 min. Carefully remove and discard the supernatant.**
5. **Repeat steps 3 and 4 twice.**
6. **Pipet 1 ml of 100% ethanol* into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 2 min.**

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

7. **Carefully remove and discard the supernatant.**
8. **Do not disturb the pellet.**
9. **Repeat steps 6 and 7.**
10. **Pipet 1 ml of 96% ethanol* into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 2 min.**
11. **Carefully remove and discard the supernatant.**
12. **Do not disturb the pellet.**
13. **Repeat steps 10 and 11.**
14. **Pipet 1 ml of 70% ethanol* into the tube containing the pellet and mix by vortexing. Incubate for 10 min. Centrifuge the tube at full speed for 2 min.**
15. **Carefully remove and discard the supernatant.**
16. **Do not disturb the pellet.**
17. **Repeat steps 14 and 15.**

    Note: If necessary, repeat the centrifugation step to enable removal of any residual ethanol. Do not disturb or remove any of the pellet.
18. **Proceed immediately with the protocol: "Extraction of Total Protein from FFPE Tissue Sections for Western Blot and RPA" on page 17.**

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

Protocol: Extraction of Total Protein from FFPE Tissue Sections for Western Blot and RPA

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, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- β-mercaptoethanol
- Disposable gloves
- Bench-top centrifuge or microcentrifuge capable of reaching 14,000 x *g*
- Vortexer
- Water bath or heating block capable of reaching 100°C
- Thermomixer® (e.g., Eppendorf, Hamburg, Germany)*

Important points before starting

- If using the FFPE Tissue Extraction Kit the first time, read the Important Notes on page 12.
- Procedures using β-mercaptoethanol should be performed in a fume hood.

Things to do before starting

- Extraction Buffer EXB Plus is supplied without β-mercaptoethanol. For each extraction procedure add 6 µl of β-mercaptoethanol to 94 µl of Extraction Buffer EXB Plus to obtain a working solution.

Table 3. Volumes added to obtain a working solution of buffer

No. extractions	1	2	3	4	5	6	7	8	9	10
Volume of Extraction Buffer EXB Plus (µl)	94	188	282	376	470	564	658	752	846	940
Volume β-mercaptoethanol (µl)	6	12	18	24	30	36	42	48	54	60

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Procedure

Protein extraction

1. **Pipet 100 µl Extraction Buffer EXB Plus supplemented with β-mercaptoethanol into the tube containing the excised tissue or pellet and mix by vortexing. Seal the Collection Tube with a Collection Tube Sealing Clip (supplied).**
2. **Incubate on ice for 5 min, and mix by vortexing.**

   Note: Be sure that Collection Tubes are properly sealed with a Collection Tube Sealing Clip before performing step 3.
3. **Incubate the tube on a heating block at 100°C for 20 min.**
4. **Using a Thermomixer, incubate the tube at 80°C for 2 h with agitation at 750 rpm.**
5. **After incubation, place the tube at 4°C for 1 min and remove the Collection Tube Sealing Clip.**

   Note: Be sure that Collection Tube Sealing Clip has been removed before starting the centrifugation step.
6. **Centrifuge the tube for 15 min at 14,000 x *g* at 4°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml Collection Tube (supplied).**

   Note: For quantification of protein yield, use the Lowry method (e.g., Bio-Rad RC DC Protein Assay Kit, cat. no. 500-0122). Dilute an aliquot of extracted protein fraction in a ratio of 1:3 with distilled water and perform the tube assay protocol according manufacturer's instructions. You may also use the Bradford method (e.g., Bio-Rad 500-0201). Dilute an aliquot of extracted protein fraction in a ratio of 1:100 with distilled water and perform the protein quantification using 100 µl of the diluted sample and 900 µl 1x Dye. If using the Bradford method, prepare a standard row by serial 2-fold dilution using the provided Bovine serum albumin (BSA) standard, resulting in the following concentrations:

Table 4. Dilution of the BSA standard for the Bradford assay

Standard no.	1	2	3	4	5	6
Concentration (µg/µl)	0.05	0.025	0.0125	0.0063	0.0032	0.0016

For SDS-PAGE analysis, add 1/5 volume 5x sample buffer to the protein lysates.

Protocol: Extraction of Proteins from FFPE Tissues and Cleanup for 2D-PAGE or MS Analysis

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, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- β-mercaptoethanol
- Ethanol
- Water (reverse osmosis- [RO-] or HPLC-grade water)
- Disposable gloves
- Bench-top centrifuge or microcentrifuge capable of reaching 14,000 x *g*
- Vortexer
- Water bath or heating block capable of reaching 100°C
- Thermomixer (e.g., Eppendorf, Hamburg, Germany)*

For MS analysis:

- RapiGest SF (Waters Corporation, cat. no. 186001860)
- Iodoacetamide (Sigma-Aldrich Chemie GmbH, cat. no. I1149)
- Trypsin (1 mg/ml Sequencing Grade Modified Trypsin; Promega Corporation, cat. no. V511A)
- DTT (Calbiochem, cat. no. 233153)
- Trifluoroacetic acid (TFA) (Sigma-Aldrich Chemie GmbH, cat. no. 91699)
- Speedvac evaporator (with rotor/adapters for 0.5 ml tubes)

Important points before starting

- Procedures using β-mercaptoethanol, heptane (steps 3–6), methanol (steps 4–6 and 13–22), and chloroform (steps 15–22) should be performed in a fume hood. All organic solvents should be disposed of according to applicable environmental regulations.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

- Unless indicated, all centrifugation steps are carried out at 20–25°C using a bench-top microcentrifuge (e.g., Eppendorf Micro Centrifuge 5417C or Hereaeus Biofuge® 15).*

Things to do before starting

- Extraction Buffer EXB Plus is supplied without β-mercaptoethanol. For each extraction procedure add 6 µl of β-mercaptoethanol to 94 µl of Extraction Buffer EXB Plus to obtain a working solution.

Table 5. Volumes added to obtain a working solution of buffer

No. extractions	1	2	3	4	5	6	7	8	9	10
Volume Extraction Buffer EXB Plus (µl)	94	188	282	376	470	564	658	752	846	940
Volume β-mercaptoethanol (µl)	6	12	18	24	30	36	42	48	54	60

Procedure

Deparaffinization

1. **Cut up to 3 serial 10–15 µm thick sections from the same block of FFPE tissue.**

   Note: If you are not sure how much protein your sample contains, we recommend using 2 sections, each with a thickness of 10 µm and an area of 100 mm^2 per preparation.
2. **Immediately place the sections in a 1.5 ml collection tube (supplied).**
3. **Pipet 0.5 ml heptane into the tube. Close tube tightly, vortex vigorously for 10 s, and incubate for 1 h at room temperature (15–25°C).**
4. **Add 25 µl methanol, close tube tightly, and vortex vigorously for 10 s.**
5. **Centrifuge the tube in a microcentrifuge at 9000 x *g* for 2 min.**

   The tissue will form a pellet at the bottom of the tube.
6. **Carefully remove the supernatant using a pipet. Discard the supernatant and air dry the pellet for 5 min.**

   Do not decant the supernatant and do not disturb the pellet.

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Protein extraction

7. **Pipet 100 µl Extraction Buffer EXB Plus supplemented with β-mercaptoethanol into the tube containing the pellet and mix by vortexing. Seal the collection tube with a Collection Tube Sealing Clip (supplied).**
8. **Incubate on ice for 5 min, and mix by vortexing.**

   Note: Be sure that collection tubes are properly sealed with a Collection Tube Sealing Clip before performing step 9.
9. **Incubate the tube on a heating block at 100°C for 20 min.**
10. **Using a Thermomixer, incubate the tube at 80°C for 2 h with agitation at 750 rpm.**
11. **After incubation, place the tube at 4°C for 1 min and remove the Collection Tube Sealing Clip.**

    Note: Be sure that Collection Tube Sealing Clip has been removed before starting the centrifugation step 12.
12. **Centrifuge the tube for 15 min at 14,000 x *g* at 4°C. Transfer the supernatant containing the extracted proteins to a new 1.5 ml collection tube (supplied).**

    Note: For quantification of protein yield, use the Lowry method (e.g., Bio-Rad RC DC Protein Assay Kit, cat. no. 500-0122). Dilute an aliquot of extracted protein fraction in a ratio of 1:3 with distilled water and perform the tube assay protocol according manufacturer's instructions. You may also use the Bradford method (e.g., Bio-Rad 500-0201). Dilute an aliquot of extracted protein fraction in a ratio of 1:100 with distilled water and perform the protein quantification using 100 µl of the diluted sample and 900 µl 1x Dye. If using the Bradford method, prepare a standard row by serial 2-fold dilution using the provided Bovine serum albumin (BSA) standard, resulting in the following concentrations:

Table 6. Dilution of the BSA standard for the Bradford assay

Standard no.	1	2	3	4	5	6
Concentration (µg/µl)	0.05	0.025	0.0125	0.0063	0.0032	0.0016

For SDS-PAGE analysis, add 1/5 volume 5x sample buffer to the protein lysates.

Preparation of protein sample for 2D-PAGE

13. **Add 400 µl methanol to 100 µl protein solution from step 12. Close tube tightly, and vortex vigorously for 10 s.**

14. **Centrifuge the tube in a microcentrifuge at 9000 x *g* for 10 s.**
15. **Add 100 µl chloroform to the tube. Close tube tightly, and vortex vigorously for 10 s.**
16. **Centrifuge the tube in a microcentrifuge at 9000 x *g* for 10 s.**
17. **Add 300 µl water, close tube tightly, and vortex vigorously for 10 s.**
18. **Centrifuge the tube in a microcentrifuge at 9000 x *g* for 1 min.**

    Note: After centrifugation, the sample separates into 3 phases: a lower, colorless, organic (chloroform) phase; a white interphase containing protein; and an upper, colorless, aqueous phase.
19. **Carefully remove and discard the upper aqueous phase.**

    Do not disturb the interphase or lower phase.
20. **Add 300 µl methanol, close tube tightly, and vortex vigorously for 10 s.**
21. **Centrifuge the tube in a microcentrifuge at 9000 x *g* for 2 min.**
22. **Carefully remove and discard the supernatant.**

    Note: The protein pellet is visible as a transparent or white gel-like pellet at the bottom of the tube.
23. **Wash the pellet by adding 1 ml ethanol and centrifuging the tube in a microcentrifuge at 9000 x *g* for 2 min. Carefully remove and discard the supernatant.**

    Note: Do not dry the pellet.
24. **Redissolve the protein pellet in an appropriate volume of a sample buffer suitable for 2D-PAGE.**

    Note: For recommended 2D-PAGE sample buffers, please refer to the Appendix on page 25. After adding sample buffer, incubate the tube for 1 h at room temperature (15–25°C) and sonicate for 60 s. Proceed with 2D-PAGE sample separation.

    Depending on the gel dimensions and staining method used, the protein amount needed for 2D-PAGE analysis varies from 50–250 µg.

Tryptic digestion of extracted proteins for mass spectrometry

1. **Dissolve the protein pellet from step 23 (desalt a volume of protein lysate that corresponds to 25 µg of extracted protein) in 10 µl of 1% (w/v) RapiGest SF in 25 mM NH_4HCO_3.**
2. **Add 10 µl of 50 mM DTT in 25 mM NH_4HCO_3.**
3. **Using a Thermomixer, incubate the tube at 37°C for 1 h with agitation at 950 rpm.**
4. **Add 10 µl of 100 mM iodoacetamide in 25 mM NH_4HCO_3.**

5. **Using a Thermomixer, incubate the tube at 37°C for 1 h with agitation at 950 rpm. Mix the tube every 10 min by vortexing briefly.**

   Note: A heating block may also be used for incubation.

6. **Add 90 µl of 25 mM NH_4HCO_3.**
7. **Add 20 µl of 0.25 µg/µl trypsin in 25 mM NH_4HCO_3. The enzyme/substrate ratio is 1:5 (w/w).**

   Note: Sequencing Grade Modified Trypsin from Promega is supplied lyophilized and must first be dissolved in the supplied reconstitution buffer according to the manufacturer's instructions to obtain a 1 mg/ml stock solution. This stock solution can be stored up to 6 months at –20°C. Prior to use, the stock solution is diluted to a concentration of 0.25 µg/µl using 25 mM NH_4HCO_3.

8. **Incubate at 37°C overnight (i.e., minimum 16 h).**
9. **Stop the digestion by adding 20 µl of 5% (v/v) TFA.**
10. **Using a Thermomixer, incubate the tube at 37°C for 1 h with agitation at 950 rpm.**
11. **Microcentrifuge at 13,000 rpm for 30 min at room temperature (15–25°C).**
12. **Transfer the supernatant containing the tryptic peptides to a new 0.5 ml microcentrifuge tube and dry under vacuum using a Speedvac evaporator with adaptors for 0.5 ml microcentrifuge tubes.**

    Note: Alternatively, use empty 1.5 ml reaction tubes without lids as adaptors.

    Note: Dried peptides should be analyzed as soon as possible. Avoid long-term storage (4 weeks or longer). Depending on your MS equipment and the downstream application dissolve your dried peptides in the solvent of your application.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists at Novus Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook (For contact information, visit www.novusbio.com).

	Comments and suggestions
Low protein yield	
a) Poor quality starting material	Fixing samples for >24 hours or storing for very long periods may prevent complete extraction of protein.
b) Too little starting material	Increase the amount of starting material.
c) Insufficient deparaffinization or too much paraffin in sample	During deparaffinization, ensure that supernatants are completely and carefully removed without disturbing the tissue pellet. If processing samples containing large amounts of paraffin, remove excess paraffin using a scalpel.

Appendix: Buffer Preparation

2D-PAGE Sample Buffer

Sample buffer (10 ml)

8 M Urea	4.8 g Urea (MW 60.06 g/mol)
60mM DTT	92 mg DTT (MW 154.25 g/mol)
4% (w/v) CHAPS	400 mg CHAPS (MW 614.88)
2% (v/v) Ampholyte	200 μl (e.g., Pharmalyte™, broad range pH 3–10, GE Healthcare cat. no. 17-0456-01)

MS lysis Buffer

Lysis buffer (1 ml)

25 mM NH_4HCO_3	19.8 mg NH_4HCO_3 (MW 79.06 g/mol)
1% (w/v) RapiGest SF	10 mg RapiGest SF (Waters, cat. no. 186002123)

DTT solution

50 mM DTT	7.7 mg DTT (MW 154.25 g/mol) in 25 mM NH_4HCO_3

Iodacetamide solution (1 ml)

100 mM Iodoacetamide	18.5 mg Iodoacetamide (MW 184.96 g/mol) in 25 mM NH_4HCO_3

Ordering Information

Product	Contents	Cat. no.
FFPE Tissue Extraction Kit(20)	For 20 protein preparations from formalin-fixed, paraffin-embedded tissue samples: Extraction Buffer, Collection Tubes, Collection Tube Sealing Clips	NBP2-37854

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