May 2008

Qproteome™ FFPE Tissue 2D-PAGE Kit Handbook

For isolation of proteins from formalin-fixed, paraffin-embedded tissue sections and sample cleanup for 2D-PAGE analysis

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Contents

Kit Contents

Qproteome FFPE Tissue 2D-PAGE Kit **Cat. no.**	**No. of preps 20** **37633**
Extraction Buffer EXB	2 x 1 ml
Collection Tubes (1.5 ml)	50
Collection Tube Sealing Clips	20
FFPE Solvent Set	1
Handbook	1

Storage

Extraction buffer EXB should be stored at –20°C upon arrival. The other components of the Qproteome FFPE Tissue 2D-PAGE 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 kit is stable for at least 12 months.

Product Use Limitations

Qproteome kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN 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, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN 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. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, 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 QIAGEN products. If you have any questions or experience any difficulties regarding the Qproteome FFPE Tissue 2D-PAGE Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN 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 QIAGEN. 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.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Qproteome kits 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 format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

The following risk and safety phrases apply to components of Qproteome FFPE Tissue 2D-PAGE Kit.

FFPE Solvent Set

Contains heptane: Highly flammable, irritant, dangerous for the environment. Risk and safety phrases:* R11-38-50/53-65-67. S9-16-29-33-60-61-62

Contains chloroform: Harmful, irritant. Risk and safety phrases:* R22-38-40-48/20/22. S36/37

Contains methanol: Highly flammable, toxic. Risk and safety phrases:* R11-23/24/25-39/23/24/25. S7-16-36/37-45

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

* R11: Highly flammable; R22: Harmful if swallowed; R23/24/25: Toxic by inhalation, in contact with skin and if swallowed;R38: Irritating to skin; R39/23/24/25: Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed; R40: Limited evidence of a carcinogenic effect; R48/20/22: Harmful: danger of serious damage to health by prolonged exposure through inhalation and if swallowed; R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; R65: Harmful: may cause lung damage if swallowed; R67: Vapors may cause drowsiness and dizziness. S7: Keep container tightly closed; S9: Keep container in a well-ventilated place; S16: Keep away from sources of ignition — No smoking; S29: Do not empty into drains; S33: Take precautionary measures against static discharges; S36/37: Wear suitable protective clothing and gloves; S45: In case of accident or if you feel unwell seek medical advice immediately (show the label where possible); S60: This material and its container must be disposed of as hazardous waste; S61: Avoid release to the environment. Refer to special instructions/safety data sheet; S62: If swallowed, do not induce vomiting: seek medical advice immediately and show this container or label.

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.

The Qproteome FFPE Tissue 2D-PAGE Kit is used for extracting proteins from formalin-fixed, paraffin-embedded (FFPE) tissue. Extraction efficiency is comparable to that seen from frozen tissue. The extracted proteins are suitable for 2D-analysis after subsequent sample cleanup.

Principle and procedure

The Qproteome FFPE Tissue procedure provides optimized conditions for extracting total protein from FFPE tissue. After deparaffinization, tissue is incubated in an optimized lysis buffer at two different temperatures in a process that reverses formalin crosslinking and untangles protein molecules. After a centrifugation step, the supernatant containing the released proteins is recovered. In the next step, the proteins are desalted, removing buffer substances and enabling the sample to be used for 2D-PAGE.

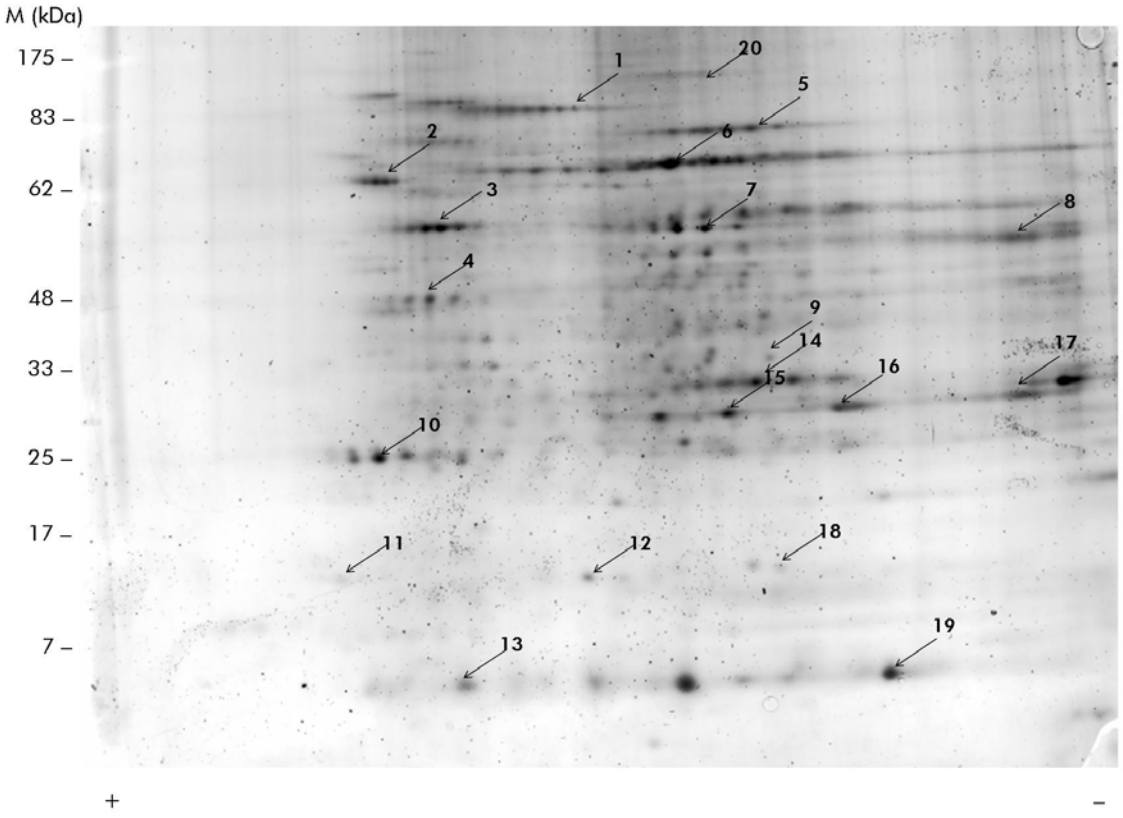
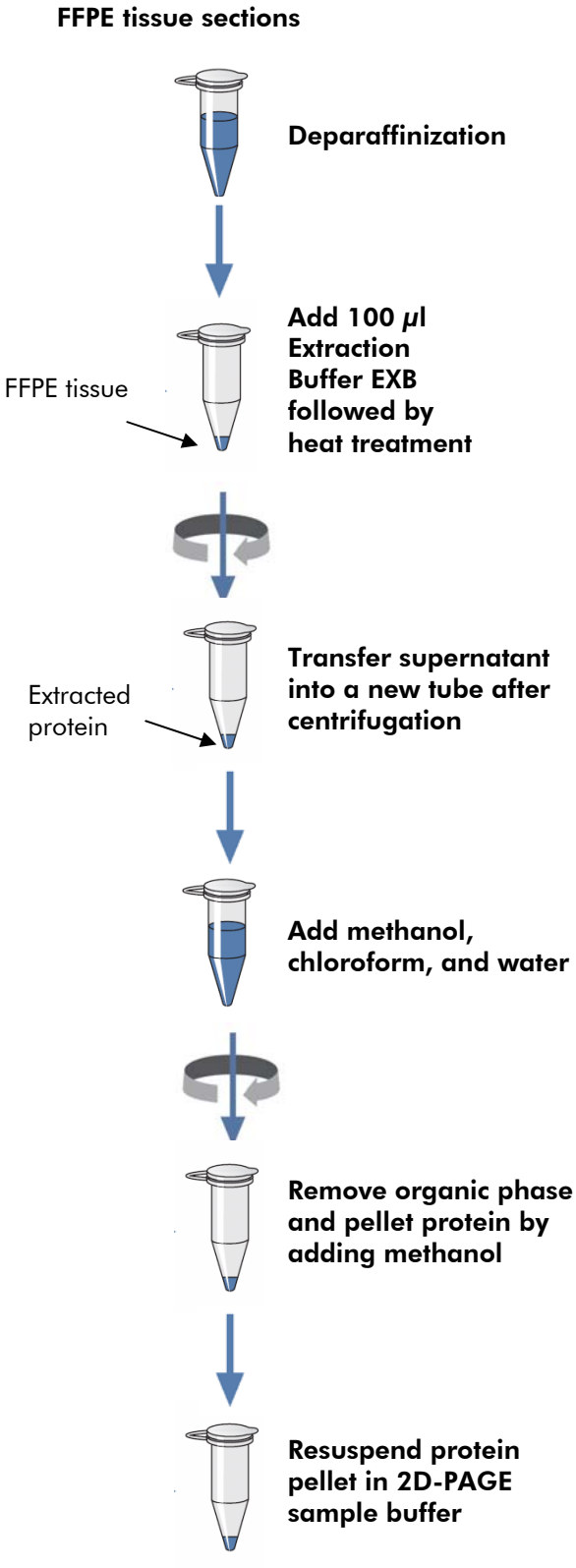


Figure 1. Efficient extraction and identification of proteins from FFPE Tissue after 2D-PAGE. Serial sections from rat liver FFPE tissue blocks were prepared using the Qproteome FFPE Tissue 2D-PAGE Kit. Extracted proteins were cleaned up prior to 2D-PAGE analysis (150 µg each) according the protocol on page 14. 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



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 (≥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
Heart (rat)*	3	~200–250	80 µg
Liver (rat)*	3	~200–250	150 µg
Colon (rat)*	3	~200–250	100 µg
Brain (rat)*	3	~150	100 µg
Kidney (rat)	3	~200–250	150 µg
Lung (rat)	3	~200–250	80 µg

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

Protocol: Extraction of Proteins from FFPE Tissues and Cleanup for 2D-PAGE 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.

- 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)*

Important points before starting

- If using the Qproteome FFPE Tissue 2D-PAGE Kit the first time, read the Important Notes on page 11.
- Procedures using 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.
- 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)*.

Procedure

Deparaffinization

1. **Cut up to three 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).**

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

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 pipette. Discard the supernatant and air dry the pellet for 5 min.**
   Do not decant the supernatant and do not disturb the pellet.

Protein extraction

7. **Pipet 100 µl Extraction Buffer EXB 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 (e.g., Bio-Rad DC Protein Assay Kit, cat. no. 500-0111) or BCA method (e.g., Pierce Micro BCA Protein Assay Kit, cat. no. 23235). Dilute an aliquot of extracted protein with the same volume of distilled water and perform the protein quantification. For SDS-PAGE/western blot analysis, add 1/5 volume 5x sample buffer. After quantification, extracted proteins can be stored for up to 1 week at 4°C. For longer-term storage, aliquot the extracted proteins and store at –20°C. Avoid repeated freeze–thaw cycles.

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 16. 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.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.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: 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)

Ordering Information

Product	Contents	Cat. no.
Qproteome FFPE Tissue 2D-PAGE Kit	For 20 protein preparations from formalin-fixed, paraffin-embedded tissue samples and subsequent cleanup for 2D-PAGE: Extraction Buffer, Collection Tubes (1.5 ml), Collection Tube Sealing Clips, FFPE Solvent Set	37633
Related products		
Qproteome FFPE Tissue Kit (20)	For 20 protein preparations from formalin-fixed, paraffin-embedded tissue samples: Extraction Buffer, Collection Tubes, Collection Tube Sealing Clips	37623

Notes

The Qproteome FFPE Tissue 2D-PAGE Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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Japan ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

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