

**Regarding the Use of Proteomics
Consignment Analysis**

Open Facility Center
20240208v1

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1. Utilization Procedure

① Meeting

A meeting is necessary to discuss the request details and sample preprocessing.

Please contact Nishioka (tomoki.nishioka@fujita-hu.ac.jp) or Tanaka (miho.tanaka@fujita-hu.ac.jp), Extension: 9814.

② Schedule Adjustment

Once the experiment schedule is determined, we will proceed with scheduling adjustments.

Samples brought in without prior notice will not be accepted.

③ Proteomics Consignment Analysis Request Form and Sample Submission

Please download the "Proteomics Consignment Analysis Request Form" from the Open Facility Center website. URL: <https://www.fujita-hu.ac.jp/~kyoriken/jyutaku/index.html>

You are required to fill out the required information and bring it with the sample.

④ Sample preparation - LC-MS measurement - Analysis using Proteome Discoverer

1 to 3 weeks (additional time may be required depending on the situation)

⑤ Return of results

The report and **analysis results (.xlsx)** will be sent via email.

Please review the received results promptly.

LC-MS measurement data (.raw) is too large to be sent via email. Please bring a virus-checked HDD to collect the data at your convenience.

*Please ensure that you back up the data yourself.

As a general rule, the analysis laboratory will store the data for 3 years, after which the data will be responsibly deleted.

2. Sample Preparation

◇ When requesting sample preparation

Proteomics sample preparation is complex and lacks standardized methods. Protocols vary depending on the type and purpose of the sample. Therefore, please find reference papers, and we will discuss and decide on the buffer and preprocessing methods used for protein extraction.

As a result of the consultation, there may be cases where we cannot accept the consignment depending on the preprocessing requirements. Thank you for your understanding.

Consignment Contents

① In-gel digestion_ SDS-PAGE

Required Sample Volume: It is possible if protein bands can be detected.

Preprocessing details: *Gel excision* / *Destaining* / Reduction and alkylation / Trypsin digestion / Desalting

② In-solution digestion: Protein extraction solution from cells or tissues

Required Sample Volume: More than 10 µg of protein (approximate guideline: 1x10⁶ cells / 10 mg tissue)

*Please perform protein quantification using methods compatible with surfactants, such as the BCA assay.

Preprocessing details: Removal of surfactants / Reduction and alkylation / Trypsin digestion / Desalting

Methods: SP3 method [Nat Protoc. 2019 Jan;14(1):68-85],

PTS method [J Proteome Res. 2008 Feb;7(2):731-40],

Methanol-chloroform precipitation method, etc.

◇ When requesting LC-MS measurement only

Surfactants and salts used for protein extraction can adversely affect LC-MS.

If you perform sample preparation yourself, please provide preparation methods during the consultation. Additionally, we generally do not accept specifications for sample injection volume.

3. Analysis Equipment

- ◇ Mass spectrometer: **Orbitrap Fusion** [Thermo Fisher Scientific]
HPLC: **EASY-nLC1000** [Thermo Fisher Scientific]
Trap column : Acclaim PepMap 100
Analytical column : EASY-Spray LC Columns
- ◇ Mass spectrometer: **Q Exactive** [Thermo Fisher Scientific]
HPLC: **Advance UHPLC** [Bruker-Michrom]
Trap column : MonoCap C18 Trap Column
Analytical column : MonoCap C18 Nano-flow 0.1x150mm
- ◇ Database search : **Proteome Discoverer 2.4** [Thermo Fisher Scientific]
Search engine : Sequest HT [Thermo Fisher Scientific] and/or Mascot [Matrix Science]

4. Proteomics Consignment Analysis Fees and Payment Methods

- ◇ Proteomics Consignment Analysis

Fees for **Sample Preparation**

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|--|----------------------|
| ① In-gel digestion: SDS-PAGE gel | 2,600 yen per sample |
| ② In-solution digestion: Protein extraction solution | 2,600 yen per sample |

LC-MS measurement

Orbitrap Fusion

- | | |
|--------------------------|-----------------------|
| ① In-gel digestion: | 9,900 yen per sample |
| ② In-solution digestion: | 13,900 yen per sample |

Q Exactive

- | | |
|--------------------------|----------------------|
| ① In-gel digestion: | 7,000 yen per sample |
| ② In-solution digestion: | 7,000 yen per sample |

- ◇ Payment Methods

We will invoice the fees monthly. We will invoice the fees monthly. Invoice requests will be sent from the Research Support Division to the departmental office around the beginning of the following month after the reception date. Payment is possible through university research funds, public research funds, etc. For inquiries, please contact the Research Support Division.

5. Disclaimer

This Consignment analysis does not guarantee the results.

Our analysis lab assumes no responsibility if analysis results are not obtained due to the quality of the submitted samples or other factors. In this case, usage fees will be invoiced.

6. Reanalysis

We will conduct the analysis with the utmost care, but please be aware that there is a possibility of sample damage due to equipment malfunctions. In the event of such damage, we will reanalyze at no additional cost. However, we cannot be held responsible for any disadvantages caused by the damage. Additionally, please submit a sufficient quantity of samples to allow for the possibility of reanalysis.

7. Research Consultation

If you have any questions about the analysis results from Proteome Discoverer 2.4, please feel free to ask. We also have a research consultation service available for technical matters such as protein extraction and protein quantification. Please feel free to use it.

Contact: Nishioka (tomoki.nishioka@fujita-hu.ac.jp),
Tanaka (miho.tanaka@fujita-hu.ac.jp)
Extension: 9814