Complex Proteomics Standard

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User Guide

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Research Use Only. Not for use in Diagnostic Procedures.

Agilent Technologies

Notices

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In this Guide...

This document describes how to use the Complex Proteomics Standard.

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

1 Before You Begin

This chapter contains information on kit components, storage conditions, and the properties of the Complex Proteomics Standard. 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 prepare the kit components and instructions for use of the standard in a sample proteomics workflow.

Contents

1 Before You Begin

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Kit contents

Table 1 Contents of the Complex Proteomics Standard kit

Materials Provided	Quantity
Pfu Protein Extract Proteomics Standard	500 µg
Proteomics Grade Trypsin	100 µg

Conditions

***Pfu* Protein Extract Proteomics Standard:** Store the lyophilized material at –20°C. After resuspension (see page 12), aliquot into single-use volumes and store the aliquots at –80°C.

Proteomics Grade Trypsin: Store the lyophilized enzyme at –20°C. After resuspension (see page 12), aliquot and store at –20°C for up to one month or at –80°C for long-term storage. Do not exceed five freeze-thaw cycles.

Required equipment and supplies

Table 2 Required Supplies

Description
Protease-free dH_20 or buffer used to prepare proteomics samples
Microcentrifuge tubes

Overview

The Complex Proteomics Standard is a complex extract of proteins from *Pyrococcus furiosus*. The standard is useful for mass spectrometry (MS)-based proteomics applications including benchmarking and validation of workflow performance (methods and instrument) and facilitating cross-experiment, cross-instrument, or cross-laboratory data comparisons.

The kit includes one vial of *Pfu* protein extract, a lyophilized total soluble extract from the hyperthermophilic archaeon *Pyrococcus furiosus*. The *P. furiosus* genome codes for approximately 2000 proteins, the majority of which are predicted to be present in the soluble protein fraction. This soluble *Pfu* protein extract represents a highly complex yet well defined mixture of proteins that allows robust testing of the full spectrum of proteomics workflow techniques. In contrast to less complex protein standard solutions currently in use, the *Pfu* protein extract contains many more proteins that cover a vast range of protein size, abundance, pI and other protein characteristics. These features make it ideal for mimicking the behavior of complex test samples. The vast genetic distance between *P. furiosus* and human or other organisms typically subjected to proteomic studies helps to avoid erroneous protein identification (e.g., in carry-over instances) and is another important consideration in the selection of *P. furiosus* as the basis for a universal, complex proteomics standard. The complete set of *P. furiosus* predicted ORFs may be downloaded from ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Pyrococcus_furiosus.

The *Pfu* protein extract, in combination with the supplied proteomics grade trypsin, has been extensively tested in MS-proteomics experiments using a variety of experimental workflows. Stringent quality-control testing and the long-term availability of a single source material are key considerations for choosing the Complex Proteomics Standard as the standard for your proteomics studies.

2 Procedures

This chapter contains information on how to prepare the kit components and instructions for use of the standard in a sample proteomics workflow.

Reconstitution of kit components

To reconstitute the *Pfu* Protein Extract Proteomics Standard

Reconstitute the vial of lyophilized *Pfu* Protein Extract Proteomics Standard to a final concentration of 10 mg/ml protein by adding 50 µl of a solution suitable for your application (see Note regarding salt content, below, before proceeding). If the lyophilized extract is dissolved in 50 µl of deionized water, the resulting solution will contain 10 mg/ml protein in 10 mM Tris (pH 8), 25 mM NaCl. Mix by gently pipetting up and down. Prepare single-use aliquots, and store the aliquots at –80°C.

NOTE The extract in each vial was lyophilized from a 50-µl solution containing 25 mM NaCl. For some proteomics applications, it may be necessary to desalt the sample after reconstitution. Common methods include using a desalting spin column or TCA-precipitation of sample proteins.

To reconstitute the trypsin

Reconstitute the Proteomics Grade Trypsin with 100 µl of 50 mM acetic acid to produce a 1 mg/ml trypsin solution. Mix by gently pipetting up and down. Prepare single-use aliquots, and store the aliquots at –20°C for up to one month or at –80°C for long term storage.

Use of the standard in proteomics experiments

For typical MS-based proteomics validation and standardization applications, the standard sample should be subjected to the same workflow designed for analysis of your samples of interest. The example workflow outlined below is used to qualify the Complex Proteomics Standard.

Example workflow for protein ID by LC/MS

1. Resuspend the vial of *Pfu* Protein Extract Proteomics Standard at a final concentration of 10 mg/ml in 50 µl of deionized water.
2. Prepare single-use aliquots, and store the aliquots at –80°C.
3. Thaw an aliquot of the standard.
4. **The extract in each vial was lyophilized from a 50-µl solution containing 25 mM NaCl. An initial desalting step, such as TCA-precipitation should be included, as needed, for your workflow.**
5. Adjust the sample to the desired protein concentration in final buffer conditions of 50 mM NH_4HCO_3 (pH 8.0), 4 mM DTT, and 50% trifluoroethanol (TFE).
6. Reduce and denature the proteins by incubating the sample at 60°C for 1 hour. Allow the sample to cool to room temperature.
7. Alkylate the proteins by adding iodoacetamide to a final concentration of 15 mM then incubating the sample in the dark for one hour at room temperature.
8. Add a sufficient amount of dilution buffer [50 mM NH_4HCO_3 (pH 8.0)] to bring the TFE concentration to less than 5%.
9. Digest the standard sample with the supplied trypsin enzyme. Add the appropriate volume of trypsin (1 mg/ml solution) such that the ratio of trypsin:protein in the sample is 1:20 to 1:50.
10. Incubate the trypsin digestion reaction at 37°C for 4 to 18 hours. Terminate the digestion reaction by adding formic acid to a final concentration of 5% (v/v).
11. Analyze the digested protein sample by mass spectrometry using the Agilent Q-TOF LC/MS platform or another suitable LC/MS platform.

To ID proteins in the standard sample, download the predicted ORF sequences for the complete *P. furiosus* genome from GenBank at ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Pyrococcus_furiosus. Consult the manual for your protein database search application for information on compatible sequence file types and on how to add the *P. furiosus* sequence data to your local protein sequence database. Sequence files obtained from different database hosts may vary in sequence data representation and quality. We recommend using the NCBI-maintained *P. furiosus* genome sequence files to ensure that data analysis is performed using the most complete and current information.

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In This Book

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