

PPS Silent® Surfactant, Acid Cleavable Detergent USE AND STORAGE INSTRUCTIONS

INTRODUCTION

PPS Silent Surfactant is a mass spectrometry compatible reagent for the extraction and solubilization of hydrophobic proteins and improvement of in-solution enzymatic digestions of proteins. PPS is an effective detergent designed to have minimal negative impact on mass spectrometry. Simply lowering the pH of the digest buffer cleaves this reagent, allowing MALDI/MS, HPLC/MS, or HPLC/UV analysis.

STORAGE AND STABILITY

Store unopened vacuum-packaged vial out of direct sunlight at -20°C or lower until the expiration date listed on the package. PPS is hygroscopic and is cleaved slowly by water at neutral pH, and at an accelerated rate at acidic or basic pH. Once the package is opened to air, the contents should be immediately reconstituted in aqueous buffer (pH 7–8), protected from elevated temperatures, and used within 12 hours.

Note: PPS Silent Surfactant is hydrolyzed when exposed to water. PPS is packaged in single-use 1 mg and 10 mg containers which are not intended to be resealed. The customer assumes all responsibility for proper handling and storage of PPS once the package is opened.

RECONSTITUTION OF PPS SILENT SURFACTANT

Shown in Table 1 are the volumes required to reconstitute a 1 mg vial of PPS in water or buffer to obtain preferred concentrations. If mass spectrometric analysis is planned, the recommended buffer is 50mM Ammonium Bicarbonate (NH_4HCO_3). Alternative buffers, such as 10 mM Tris-HCl or 25 mM sodium phosphate, are also PPS compatible.

The recommended concentration is 0.1–0.2% (w/v) PPS. Higher PPS concentrations may be used for increased solubility of hydrophobic molecules.

Solubility of zwitterionic PPS Silent Surfactant in organic solvents is limited and should be tested on an individual basis.

Note: PPS Silent Surfactant is sold in 10 mg vials for applications requiring large volumes of detergent solution. PPS must be transferred to a larger container to accommodate the larger volume of buffer necessary to reach the desired concentration.

TABLE 1. RECONSTITUTION OF PPS SILENT SURFACTANT POWDER

VOLUME OF BUFFER ADDED PER 1 MG PPS	PPS CONCENTRATION (w/v)
1 ml	0.1%
500 μL	0.2%
200 μL	0.5%
100 μL	1%
50 μL	2%

RECOMMENDED PROTEIN DIGESTION PROCEDURE FOR COMPLEX MIXTURES

MATERIALS NEEDED

- PPS Silent Surfactant (Part # 21011, 1 mg vial; Part # 21010, 10 mg vial)
- 50mM Ammonium Bicarbonate buffer
- 500mM DTT
- 500mM IAA (Iodoacetamide – light sensitive)
- 500mM HCl
- 250 ng/ μl Trypsin, modified, sequencing grade in 0.01% acetic acid
- 100 mM CaCl_2

RECOMMENDED PROCEDURE

- Make 0.2% PPS diluted in 50 mM Ammonium Bicarbonate pH 7.8 (1 mg surfactant per 500 μl 50 mM Ammonium Bicarbonate pH 7.8).
- Using low adhesion microcentrifuge tubes, add 100 μl 0.2% PPS per 100 μl protein mixture (1:1) [final concentration of PPS should be 0.1% (w/v)]. If protein is in pellet, add 25–50 μl of 0.1% PPS.
- Vortex the sample.
- Add DTT to a final concentration of 5mM.
- Incubate sample at 50°C for 30 minutes.
- Cool the sample to room temperature.
- Add IAA to a final concentration of 15mM.
- Place sample in dark at room temperature for 30 minutes.
- Add CaCl_2 to a final concentration of 1mM.
- Add trypsin for a final concentration of 1:50 enzyme:protein. If total amount of protein is very low just add 1–2 μg of trypsin.
- Incubate 4h with shaking at 37°C .

Note: When dealing with hydrophobic or proteolytically resistant proteins, the optimum PPS concentration and digestion time may vary.

PPS SILENT SURFACTANT CLEAVAGE

Once the sample has been prepared, reconstitute or extract the sample containing the protein(s) of interest in PPS solution. Cleave PPS Silent Surfactant using one of the following methods:

METHOD 1: PPS HYDROLYSIS

- Prior to mass spectrometry run, add HCl to a final concentration of 250mM.
- Allow the cleavage reaction to proceed for one hour at room temperature.

METHOD 2: PPS HYDROLYSIS AND COMPLETE REMOVAL

- Cleave PPS and remove detergent components by dialyzing the sample of interest against 3:2:1 water/2-propanol/formic acid (pH 1.4) for 2 hours.

HPLC/MS ANALYSIS

- Spin sample and separate supernatant from the pellet if necessary (e.g. approximately 16,000 \times g, for 10 minutes).
- Proceed with LC-MS analysis of the supernatant.

Note: This procedure is also applicable for other analytical instrumentation such as HPLC/UV analysis.

REORDERING INFORMATION

PPS Silent Surfactant is available in 1 mg and 10 mg vials.

To place an order for PPS Silent Surfactant, please contact Protein Discovery by phone, fax, or e-mail.

T (865) 521-7400 / F (865) 521-3548 / sales@proteindiscovery.com

DESCRIPTION	PART NUMBER
PPS Silent Surfactant, 1 mg vial, 5-pack	21011
PPS Silent Surfactant, 10 mg vial	21010

Protein Discovery, Inc.
 418 S. Gay Street, Suite 203
 Knoxville, TN 37902
 www.proteindiscovery.com