



PPS SILENT® SURFACTANT

PROTEIN PREP REAGENTS

Use & Storage Instructions

RECOMMENDED PROTEIN DIGESTION PROCEDURE FOR COMPLEX MIXTURES

- 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 to 50 µl of 0.1% PPS.
- Vortex the sample.
- Add DTT to a final concentration of 5 mM.
- Incubate sample at 50°C for 30 minutes.
- Cool the sample to room temperature.
- Add IAA to a final concentration of 15 mM.
- Place sample in dark at room temperature for 30 minutes.
- Add CaCl₂ to a final concentration of 1 mM.
- Add Agilent Proteomics Grade 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 4 hours with shaking at 37°C.

Note:

For hydrophobic or proteolytically resistant proteins, the optimum PPS concentration and digestion time may vary.

MATERIALS NEEDED

- PPS Silent Surfactant (Part # 400500, 1 mg vials; Part # 400501, 10 mg vial)
- 50 mM ammonium bicarbonate buffer (pH 7.8)
- 500 mM DTT
- 500 mM IAA (iodoacetamide – light sensitive)
- 500 mM HCl
- Agilent Proteomics Grade Trypsin (Part # 204310, 100 µg vial) diluted to 250 ng/µl
- 100 mM CaCl₂

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 250 mM.
- 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 × 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.

INTRODUCTION

PPS Silent Surfactant is a mass spectrometry compatible detergent for the extraction and solubilization of hydrophobic proteins and improvement of in-solution enzymatic digestion of proteins.^{1,2,3}

STORAGE AND STABILITY

Store unopened vacuum-packaged vials out of direct sunlight at –20°C or lower. 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), stored at 4°C, and used within one week.

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 50 mM ammonium bicarbonate (NH₄HCO₃). Alternative buffers, such as 10 mM tris-HCl or 25 mM sodium phosphate, are also PPS-compatible. The recommended concentration is 0.1 to 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.

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%

TABLE 1.
Reconstitution of PPS Silent Surfactant powder

REFERENCES

1. Chen, E. I., McClatchy, D., Park, S. K. and Yates, J. R., 3rd (2008) *Anal Chem.* 80(22):8694-701.
2. Lu, B., McClatchy, D. B., Kim, J. Y. and Yates, J. R., 3rd (2008) *Proteomics.* 8(19):3947-55.
3. Chen, E. I., Cociorva, D., Norris, J. L. and Yates, J. R., 3rd (2007) *J Proteome Res.* 6(7):2529-38.

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Printed in USA, January, 2009

Description	Qty	Cat Nos.
PPS Silent Surfactant	1 mg vials, 5-pack	400500
PPS Silent Surfactant	10 mg vial	400501

ORDERING INFORMATION:

United States and Canada
Order: 800 424 5444 x3
Technical Services: 800 894 1304 x2

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400500-12
Revision A