protea®
Product Information Document

Progenta™ Anionic Acid Labile Surfactant

Catalog Number: ALS-100, ALS-110

PRODUCT DESCRIPTION:

Progenta Anionic Acid Labile Surfactants (AALS) are novel, acid cleavable detergents that are fully compatible with mass spectrometry analysis. The Progenta AALS's have been engineered to provide a safe, robust alternative to detergents (e.g. sodium dodecyl sulfate and Triton X) that are commonly used in proteomics work, but that negatively impact subsequent analysis by mass spectrometry. While SDS and other detergents can improve protein solubility, they can be very difficult to remove during sample prep and purification of the protein sample. These bound detergents can cause significant impairment of protein analysis by mass spectrometry, as the surfactants can suppress analyte ion signal, promote analyte adduct formation, and present as contaminants during the analysis.

The Progenta Anionic Acid Labile Surfactants are fully mass spec compatible and alleviate the problems commonly associated with SDS and other detergents in proteomics studies. At neutral pH, the Progenta AALS functions as a powerful detergent for use in sample preparation, protein solubilization, and cell lysis protocols. After completing the experimental work, the solution is adjusted to a pH of 2.5 – 3 with trifluoroacetic acid (TFA) and incubated for 10 minutes to fully cleave the AALS into small organic molecules that do not exhibit surfactant activity or interfere with analysis by mass spectrometry. Other commercially available acid labile surfactants require incubation in harsher acidic environments of pH 1.0 to 2.0 for up to several hours and may produce an oily pellet or film from the cleavage by-products. Progenta AALS has been specially engineered to provide powerful surfactant performance with the added benefit of quick, simple acid-based detergent cleavage and removal for reproducible results in mass spectrometry-based proteomics studies.

PROTOCOL:

Reconstitution of Anionic Acid Labile Surfactant (AALS)

The AALS working stock can be made up in several different solvents for subsequent usage. Recommended solvents include water, 0-50% methanol, 0-50% acetonitrile, 5-50 mM ammonium bicarbonate buffer, 5-50 mM Tris-HCl buffer, 5-50 mM sodium phosphate buffer, and 5-50 mM ammonium acetate buffer. It is important that the solvent pH be between 7 and 10 to prevent degradation of the surfactant, so the pH of each solvent should be checked prior to usage.

Table 1 shows the volumes required to reconstitute 1 mg and 5 mg vials of the AALS powder. The recommended working concentration for using the AALS is 0.01 to 1.0%. Surfactant cleavage will occur within 10 minutes in a 1% TFA solution (water-based solvent).

Table 1. Reconstitution of Anionic Acid Labile Surfactant (AALS) Powder

1mg AALS Powder		5mg AALS Powder	
Volume of Buffer to Add to AALS Powder	AALS Concentration (w/v in g/mL)	Volume of Buffer to Add to AALS Powder	AALS Concentration (w/v in g/mL)
1.0 mL	0.1%	5.0 mL	0.1%
500 µL	0.2%	2.5 mL	0.2%
200 µL	0.5%	1.0 mL	0.5%
100 µL	1.0%	0.5 mL	1.0%

General Usage Considerations

The Progenta Anionic Acid Labile Surfactant functions as an SDS analog, and it can be used in any experiment where SDS is routinely used. For most methodologies, we recommend a working concentration of 0.01-1.0% AALS. In a solution phase experiment, 1.0% trifluoracetic acid (TFA), pH 2.5-3.0 will efficiently cleave the AALS detergent within 10 minutes to produce degradation by-products that do not possess surfactant properties, do not bind to the sample or reversed phase media, or interfere with mass spectrometry analysis.

Protocol for Protein Digestion Using the Progenta Anionic Acid Labile Surfactant

Materials Needed:

- Progenta Anionic Acid Labile Surfactant (Cat. No. #ALS-100-5mg, #ALS-110-5mg, or #ALS-120-5mg)
- 50 mM ammonium bicarbonate buffer, pH 7.8
- 500 mM dithiothreitol (DTT)
- 500 mM iodoacetamide (IA)
- 10% trifluoracetic acid (TFA)
- trypsin, mass spectrometry grade (250 ng/µL in 0.01% acetic acid)

Digestion Protocol:

- Make a 0.025% AALS solution in 50mM ammonium bicarbonate, pH 7.8 (digestion buffer).
- Reconstitute the protein sample directly in the 0.01% AALS solution in a microcentrifuge tube.
  - o If the protein sample is already in a solution format, then a 0.05% AALS solution in 50 mM ammonium bicarbonate can be made and used to dilute the protein sample directly in a 1:1 ratio.
  - o If the protein sample is in a pellet form and difficult to dissolve, additional digestion buffer can be used to help solubilize the sample.
  - o For difficult to digest protein samples, a higher concentration (e.g. 0.1%) of AALS can be used.
- Vortex or sonicate the sample for 10 seconds.
- Add DTT to a final concentration of 5 mM.
- Incubate the sample at 55° C for 30 minutes.
- Cool the sample to room temperature.
- Add IA to a final concentration of 25 mM.
- Incubate the sample at room temperature in the dark for 30 minutes.
- Add trypsin for a final concentration of 1:50 (enzyme:protein).
  - o CaC_{l2} may be added to a final concentration of 1 mM to improve tryptic digestion.
- Incubate at 37° C for 4-8 hours with shaking.
  - o Proteolytically resistant proteins may require overnight digestion.

Protocol for Hydrolytic Cleavage of the Progenta Anionic Acid Labile Surfactant

- Prior to analysis by mass spectrometry, add trifluoracetic acid (TFA) to a final concentration of 1%. (e.g. Add 10 µL of 10% TFA to 90 µL of enzymatic digestion).
  - o The cleavage by-products of the AALS detergent produce a small sulfate salt. To prevent signal suppression in the mass spectrometer, the peptide sample should be desalted either by use of a functional pipette tip (e.g. C_{18} ProteaTip) or during standard desalting on a column in an LC-MS experiment.
- Allow the surfactant cleavage to proceed for 10 minutes at room temperature.
- If buffered solutions are used (e.g. ammonium bicarbonate), they may require a slightly longer time for surfactant cleavage if the final pH is >3. Additional TFA can be added to bring the pH to 2.5 – 3.0 to improve the cleavage kinetics of the hydrolysis reaction.
- Elevating the reaction temperature (20-50° C) will improve the cleavage kinetics.
- Lowered reaction temperatures (4-20° C) can be used, but the slower cleavage kinetics may require longer reaction times (10-30 minutes).

SPECIFICATIONS:

Progenta Surfactant	Catalog Numbers	Critical Micellular Concentration (CMC)	Recommended Concentrations
AALS I	ALS-100-5mg	7.7 mM	0.01 – 2.0%
AALS II	ALS-110-5mg	1.9 mM	0.01 – 2.0%
AALS III	ALS-120-5mg	23.5 mM	0.01 – 2.0%

PRODUCT STORAGE:

The Progenta Anionic Acid Labile Surfactants are provided in convenient, lyophilized 5 mg quantities. When stored unopened at -20° C, the AALS's have a shelf life of 2 years. The AALS's are hygroscopic and packaged in an argon atmosphere to prevent water absorption and subsequent degradation. At neutral pH, the AALS's will slowly hydrolyze. Under increasingly acidic conditions, as well as with elevated temperatures, the AALS's will hydrolyze more quickly. The AALS stock solution should be made in a non-protic, neutral or slightly basic pH solvent (pH 7 to 10) to prevent degradation. Any unused AALS solution should be stored at 4° C and used within 4 weeks of reconstitution.

ORDERING INFORMATION:

Progenta Anionic Acid Labile Surfactant

Cat. No.	Size
ALS-100-5mg	1 vial
ALS-100-5x5mg	5 x 5mg
ALS-100-10x5mg	10 x 5mg

Progenta Anionic Acid Labile Surfactant II

Cat. No.	Size
ALS-110-5mg	1 vial
ALS-110-5x5mg	5 x 5mg
ALS-110-10x5mg	10 x 5mg