

This procedure is used routinely in our laboratory to prepare peptides for protein sequence analysis and protein identification procedures using mass spectrometry. The method is designed for Coomassie stained proteins and is successful, in our hands, in more than 90% of experiments. We recommend that you start with a minimum of 50 pmol of stained protein for peptides destined for Edman sequence analysis and >10 pmol of protein for mass spec analysis. Clearly, the more the better. We further recommend that you have the protein in a single gel slice to reduce the amount of acrylamide present. Your goal is to obtain a high protein/acrylamide ratio for maximal peptide retrieval. This procedure can also be applied to proteins that are stained with silver IF a non-fixing method was employed. We routinely use a method published by Blum (see references and Gel Staining) which is reversible. The protocol to destain (Blum) silver-stained gels is included at the bottom of this page. In our hands, destaining silver stained proteins can double the amount of peptides retrieved from digestion.

Reduction and alkylation is considered optional. Many labs omit these steps from their procedures. Our experience has been that enough peptides are retrieved for mass spec or Edman sequence analysis if it is omitted and, often, the additional steps actually reduce recoveries and increase contamination. For this protocol, as well as all others, use the highest quality reagents and chemicals available. Wear powder-free gloves as keratins from your skin are common contaminants in these experiments.

We routinely use Promega modified trypsin for digests but also recommend Endo Lys C (EKC) (Waco Chemical Co). EKC cleaves only after Lysines and, therefore, produces larger peptides than trypsin.

This is a modification of a method published by Williams and Stone (see references).

REDUCTION AND ALKYLATION (OPTIONAL)

Dice gel slice with a fresh razor blade to bits ~2 mm x 2 mm

Wash gel slice 30' in 1 ml 30% MeOH, discard wash

Wash gel slice 60' in 500 μ l 100mM ammonium bicarb, discard wash

Add 160 μ l 100 mM ammonium bicarb that contains 2.5 mM DTT (2.5 μ l 1M DTT/ml bicarb), inc 30' at 60C

Add 10 μ l 100 mM iodoacetamide (18.5 mg/ml in water; final [] = 5.8 mM), inc RT 30' IN DARK, discard solution

DIGESTION

Add 500 μ l gel wash buffer (50% ACN/50 mM Tris pH 9.2 (for Endo-Lys C) or 50% ACN/50 mM Bicarb (for Trypsin), 60' RT, discard wash

Wash gel slice with 50 μ l ACN, let stand 10', discard

Speed Vac to dryness without radiant cover (~15 min)

Add 10 μ l of 100 mM ammonium bicarb containing 0.2 μ g Promega modified Trypsin or 10 μ l 100 mM Tris pH 9.2 containing 0.2 μ g of Waco EndoLysC; allow to absorb

Add additional 100 mM ammonium bicarb or 100 mM Tris pH 9.2 (without enzyme) to allow slices to swell to original size plus 20 μ l additional

Incubate overnight at 40C

EXTRACTION

Add 150 μ l 0.1% TFA, 60% ACN, 60' rocking at RT

Remove supernatant to a siliconized 0.5 ml eppendorf-style tube

Re-extract the slices with 150 μ l 0.1% TFA, 60% ACN as above

Combine supernatants

DESTAINING SILVER STAINED GEL SLICES (for Blum silver stained gels only)

Solutions needed: 30 mM potassium ferricyanide; 98 mg/10 ml water
(make fresh) 100 mM sodium thiosulfate; 248 mg/10 ml water

Procedure:

mix solutions together 1:1 (this is working solution)

Pipet 50 μ l of working solution onto gel slice(s)

Incubate until slice is destained (several minutes); stop with water; discard

Cover with 250 μ l of 100mM ammonium bicarb, 20 min; discard

REFERENCES:

Blum, H., Beier, H., and Gross, H.J. (1987) *Electrophoresis* 8, 93-99

Stone, K.L. and Williams, K.R. (1993) *In A Practical Guide to Protein and Peptide Purification for Microsequencing*, 2nd ed. (P. Matsuidaira, ed.) pp. 43-69. Academic Press, San Diego.