

Mass Spectrometry

Blum Silver Staining Protocol

Introduction

An important part of proteomics is the ability to analyze and identify proteins from electrophoretic gels at high throughput and sensitivity. Proteins separated by gel electrophoresis can be visualized using a number of staining methods, however only some of these are compatible with protein digestion and mass spectrometric analysis. Silver staining is sensitive, but so far only a few protocols are compatible with digestion and mass spectrometry.

Use of silver stained spots for digestion and mass spectrometry is a controversial topic. Some laboratories have difficulties obtaining a high staining sensitivity using these protocols. It is frequently discussed whether the peptide recovery from Coomassie stained spots is higher than from silver stained spots. Here we present our best protocol for sensitivity and compatibility with mass spectrometry.

Procedure

1. Fix gel in 40% EtOH, 10% HAc for 1 hr.
2. Wash gel in 30% EtOH, 2 x 20 mins.
3. Wash gel in H₂O for 20 mins.
4. Sensitize gel in 0.02% Na₂S₂O₃ for 1 min.
5. Wash gel in H₂O, 3 x 20 secs.
6. Incubate gel in cold 0.1% AgNO₃, 20 mins. at 4°C
7. Wash gel in H₂O, 3 x 20 secs.
8. Change gel chamber
9. Wash the gel in H₂O for 1 min.
10. Develop gel in 3% Na₂CO₃, 0.05% formalin
Observe the color and change solution when the developer turns yellow.
Terminate when the staining is sufficient.
11. Wash the gel in H₂O for 20 secs.
12. Terminate staining in 5% HAc

13. Wash the gel in H₂O, 3 x 10 mins.

14. Leave the gel at 4°C in 1% HAc

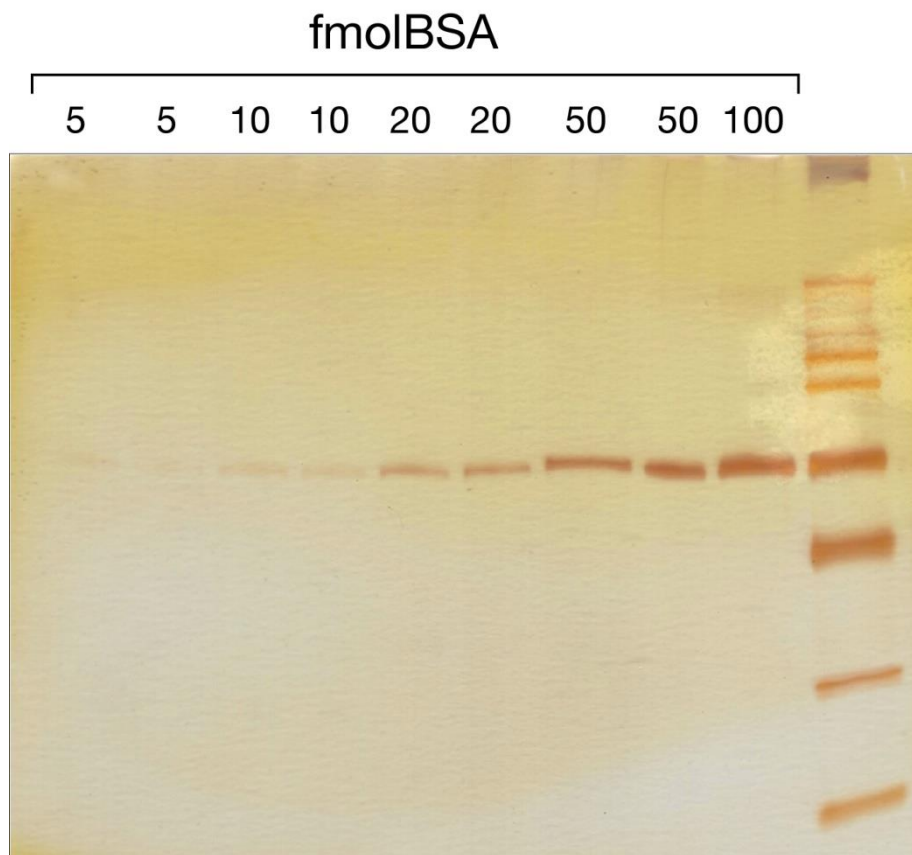


Fig. 1: SDS-PAGE from BSA at the indicated concentrations.

BSA	Mascot score	Sequence coverage
5 fmol	603	26
10 fmol	804	29
20 fmol	1092	45
50 fmol	1572	56
100 fmol	2117	74

Table 1. Mascot score and sequence coverage from indicated concentrations of BSA after database analysis. In Mascot, the score for an MS/MS match is based on the absolute probability (P) that the observed match between the experimental data and the database sequence is a random event. The reported score is $-10\log_{10}(P)$.