Several Issues regarding Silver Stained Gel Bands

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Introduction

Many users are concerned about the sensitivity of our instruments. Frequently, they would like to know the protein ID of a silver-stained gel band from immuno-preparation experiment. Although it is demonstrated that our instrument is sensitive enough to detect sub-femtomolar level of peptides, these sensitivity tests do not directly translate to the detectability of proteins in a gel band as multiple sample manipulation steps required by in-gel digest will cause significant sample loss (especially when dealing with minute amount of the sample). Although we routinely identify proteins from user submitted barely visible silver-stained bands, we are still concerned about the different methods of silver staining and their effect to detection sensitivity using mass spectrometry. Here, using 5ng BSA as a test case, we tested the sensitivity of our instrument in regarding the silver stained gel bands and tried to answer several frequently asked questions regarding preparation of silver-stained proteins for mass spectrometry analysis. Silver stain kit from PIERCE (SilverSNAP) was used in this experiment. But silver stain kits from other manufactures (specified to be mass spectrometry compatible) might also apply.

Detection Limit and Recovery for In-gel Digest of Silver Stained Gel Bands

BSA: 5 ng/well

NuPAGE Novex Bis-Tris Mini Gel, 1mm thickness (Invitrogen). Silver staining with SilverSNAP (PIERC)

Cut out the gel bands, In-gel digest, LC/MSMS.

In-gel-digest

Sequence Coverage

BSA: 5ng, In-gel-digest

Red areas indicate identified peptides

BSA: 5ng, solution digest

Red areas indicate identified peptides

Conclusion-1

• From silver stained gel band of 5ng of BSA, 7 peptides were identified, sequence coverage is 13%.
• For 5ng of BSA, the peptide recovery of in-gel-digestion is about 8-10%. 90% of the protein/peptides were possibly lost in the gel or on the tubes.
• These results are proximate and may vary slightly from different experiments.
• Our sample prep and LC-MSMS system is sensitive enough for detecting silver-stained gel bands from 5ng BSA or less.

To De-Stain or Not to De-Stain

Some silver-stain protocols for MS ask for a de-stain step prior to in-gel-digest. However, the benefit of de-stain is not clear. Here we cut two silver stained 5ng BSA bands (see left). One was de-stained based on manufacturer’s instruction and the other one was not de-stained (ctrl). Both samples were then digested with trypsin and analyzed by LC-MS.

De-stained

Sequence Coverage

BSA: 5ng, Silver Stain, No De-Staining

Red areas indicate identified peptides

BSA: 5ng, Silver Stain and De-Stamped

Red areas indicate identified peptides

Conclusion-2

• De-staining step does not improve the sensitivity of the LC/MSMS.

Effect of Over-Development of Silver Staining on Mass Spectrometry Sensitivity

To test how over-development of silver staining on mass spectrometry, we ran identical gels of 5ng BSA per lane, one gel was developed for 2 minutes (normal time needed to see the band). The other one was over-developed for 10 minutes. Both samples were then in-gel digested and analyzed by LC-MSMS.

Developed for 10 min

Developed for 2 min

Conclusion-3

• For silver stain, over development dramatically reduces the detection sensitivity using mass spectrometry.
• It is thus suggested that you stop the development immediately when the bands you intended to see are visible.

Summary

• Using PIERCE SilverSNAP kit, the detection limit should be under 5ng of protein/gel band.
• De-staining does not make any improvement for the result of LC/MSMS.
• Over-staining will hurt the sensitivity for the mass spectrometry.