



SilverQuest™ Kit

for Mass Spectrometry-Compatible Silver Staining



With the SilverQuest™ Kit, you'll get:

- Increased staining sensitivity
- Optimized reagents for improved mass spectrometry results
- Color-coded solutions for easy staining

The ultimate in silver staining performance

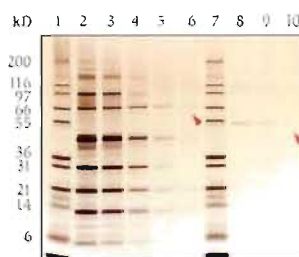


The SilverQuest™ Silver Staining Kit provides a rapid and sensitive method for silver staining proteins in polyacrylamide gels. With the SilverQuest™ Kit, you can detect as little as 0.3 ng of protein in just 30 minutes. In addition, high-performance reagents ensure complete compatibility with your mass spectrometry analysis. With the SilverQuest™ Kit, you'll get optimal staining results and have confidence in the accuracy of your mass spectrometry results.

Maximum silver staining sensitivity and speed

The SilverQuest™ Kit is based on a highly sensitive chemical reaction that involves the reduction of silver ions to metallic silver on a protein band. In order to improve (sensitize) the ability of the protein bands to react with the silver ions, a sensitizing solution is required. The sensitizer in the SilverQuest™ Kit is specially formulated to provide you with sensitive staining results in a minimal amount of time. Using the SilverQuest™ Kit's optimized microwave staining protocol (Figure 1), you'll be able to detect 0.3 ng of protein in just 30 minutes (Figure 2)—that's at least three times faster than traditional silver staining protocols. You'll get the sensitivity you need and save hours of time.

Figure 2 – Sub-nanogram sensitivity with the SilverQuest™ Kit



The samples listed below were run on a NuPAGE™ Novex Bis-Tris 4–12% Gel and then stained with the SilverQuest™ Kit. Arrows indicate 0.3 ng protein.

Lanes 1,7: 5 µl reduced Mark12™ Standard diluted 1:5
Lanes 2,3,4,5,6: 5 µl reduced protein mix diluted 1:5, 1:25, 1:100, 1:600, and 1:1100, respectively
Lanes 8,9,10: 1.2, 0.6, and 0.3 ng non-reduced BSA, respectively

Figure 1 – Overview of the SilverQuest™ microwave staining protocol

Following electrophoresis, the gel is fixed, sensitized (A), and stained (B) using the microwave protocol and kit reagents. The gel is subsequently developed (C) and stopped (D). The entire microwave protocol takes only 30 minutes to complete. Silver stained bands can then be excised (E) and destained in preparation for mass spectrometry analysis (F).



Mass spectrometry-compatible staining reagents

Silver staining of proteins followed by mass spectrometry analysis is a sensitive technique used for protein identification in proteomics. In order to determine exact mass, it is imperative that proteins be in their natural state or that modifications be accounted for during analysis by Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI/MS). Unfortunately, traditional silver staining kits contain reagents that can modify proteins and jeopardize protein identification. The SilverQuest™ Kit ensures you get the accurate results you need by eliminating the reagents that can interfere with mass spectrometry analysis. The optimized staining reagents in the SilverQuest™ Kit include:

- *A unique, glutaraldehyde-free sensitizer for improved mass spectrometry sample analysis*

For optimal mass spectrometry analysis, it is important to obtain complete trypsin digestion of your protein sample. Traditional silver staining kits contain glutaraldehyde or formaldehyde-based sensitizers. Glutaraldehyde can modify lysine residues and prevent complete trypsin digestion (1). Additionally, glutaraldehyde reduces the efficiency of protein

extraction from the gel by crosslinking two lysine residues (1). The optimized sensitizer included in the SilverQuest™ Kit does not contain glutaraldehyde. You'll obtain improved trypsin digestion and extraction of peptides from your gels.

- *Destaining solutions remove silver from the gel for sensitive detection*

Silver ions have been reported to inhibit trypsin digestion of protein (2). These ions reduce the sensitivity of detection and subsequent mass spectrometry coverage of your protein. The SilverQuest™ Kit includes destaining solutions that effectively remove silver ions from protein bands in polyacrylamide gels and improve mass spectrometry coverage of your proteins (Table 1)(3).

The SilverQuest™ Kit is the only silver staining kit that provides reagents specifically designed to make silver staining compatible with mass spectrometry analysis. You'll get enhanced trypsin digestion and efficient removal of interfering silver ions, providing you with accurate results.

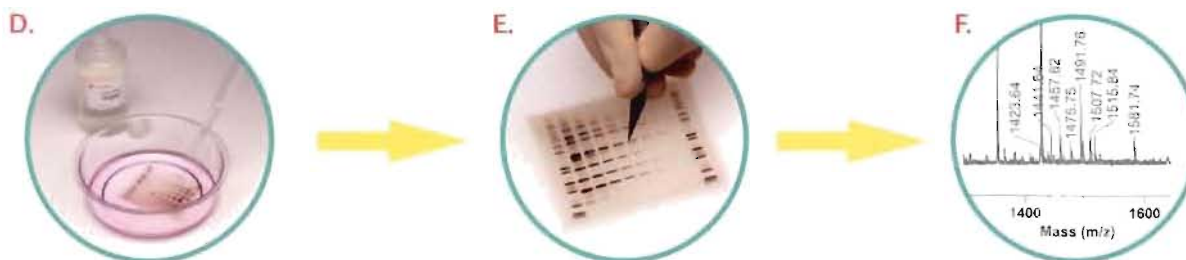
Table 1 – Mass spectrometry results after staining and destaining with the SilverQuest™ Kit

Protein	No. of peptides observed [†]	% coverage [*]	Z-value [‡]
Bovine serum albumin (BSA)	32	41	2.35
L-glutamate dehydrogenase	52	48	2.39
Phosphorylase B	31	34	2.37

[†] Number of peptide fragments resulting after trypsin digestion of 5 pmol of protein.

^{*} Percent coverage refers to the percent of protein sequence that has been identified. Typical mass spectrometry percent coverage results obtained after staining with other silver stains range from 27-30%.

[‡] Z value refers to the degree of similarity between a sample and a searched database match. A Z-value ≥ 1.65 is considered a properly identified match.



Colored reagents for simplified staining

Silver staining can be a long, labor-intensive process. The SilverQuest™ Silver Staining Kit contains color-tinted solutions (Figure 3) to provide simplified staining. You'll be able to easily identify where you are in the staining procedure, eliminating the chance

of unnecessarily repeating steps or accidentally missing one. With the SilverQuest™ Kit you can be assured of successful staining results without wasting time repeating procedures.

Figure 3 – Color tinted reagents in the SilverQuest™ Kit



Kit Component	Solution Color
Sensitizer	Orange
Stainer	Clear
Developer	Pink ¹
Developer Enhancer	Clear
Stopper	Clear
Destainer A	Yellow
Destainer B	Clear

¹Changes to clear upon addition of stopper.

Sensitive, mass spectrometry-compatible silver staining

For fast, sensitive silver staining and mass spectrometry results you can rely on, try the SilverQuest™ Kit. It's the first and only mass spectrometry-compatible silver staining kit available. Call Invitrogen and order today.

Product	Quantity	Cat. no.	Price
SilverQuest™ Kit	1 kit*	LC6070	\$160

*Sufficient reagents are supplied to stain 25 mini-gels.

References:

- 1 Rabilloud, T. (1990) *Electrophoresis* 11: 785-794.
- 2 Chambers, J.L. et al. (1974) *Biochem. and Biophys. Res. Commun.* 59: 70-74.
- 3 Gharahjadhii, F. et al. (1999) *Electrophoresis* 20: 601-605.

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