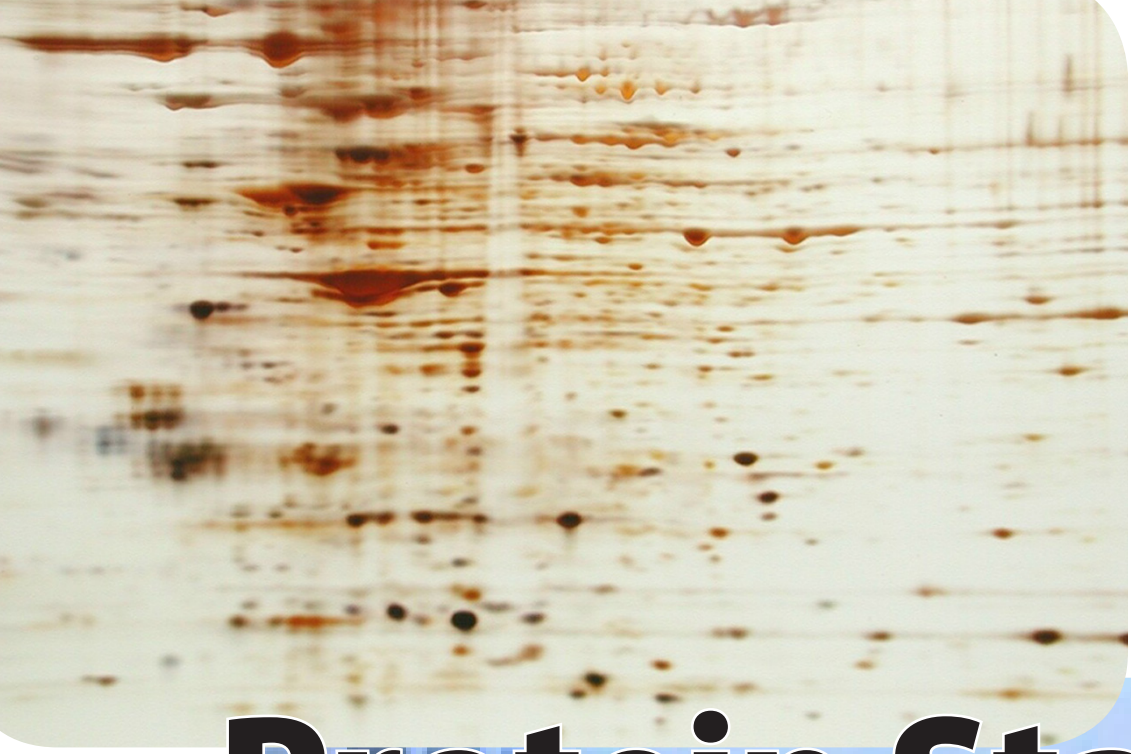
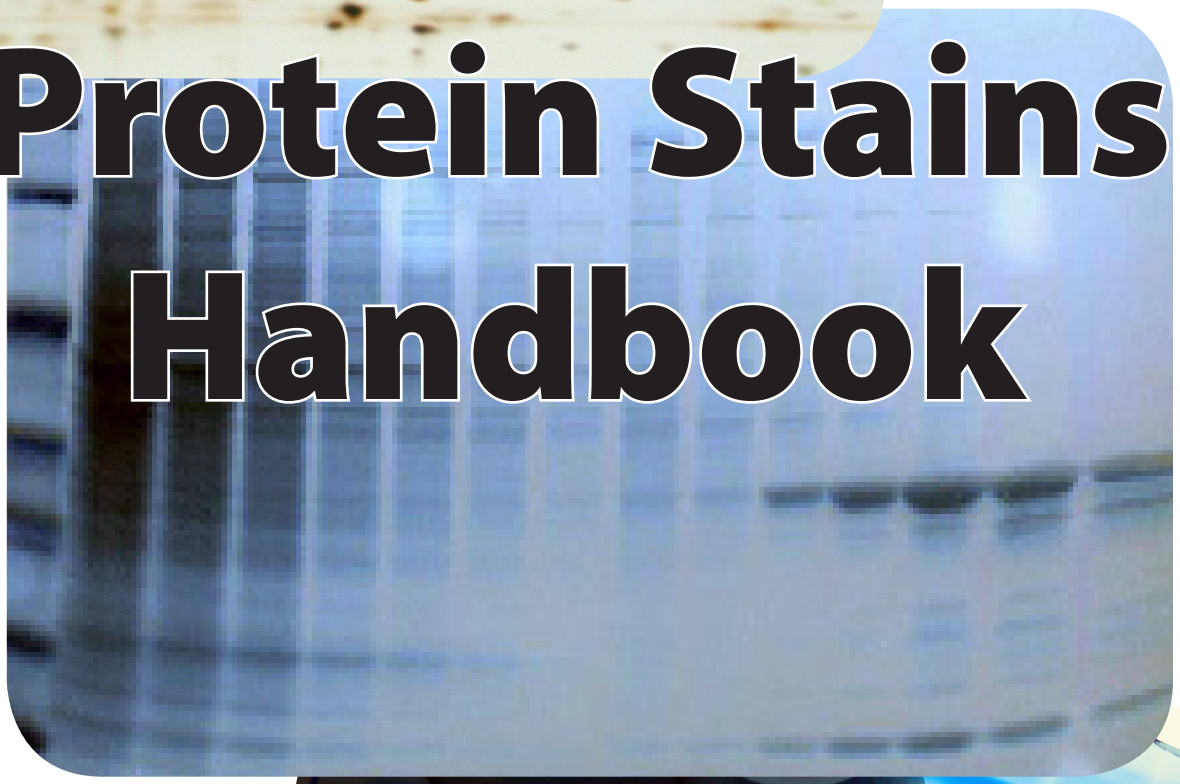
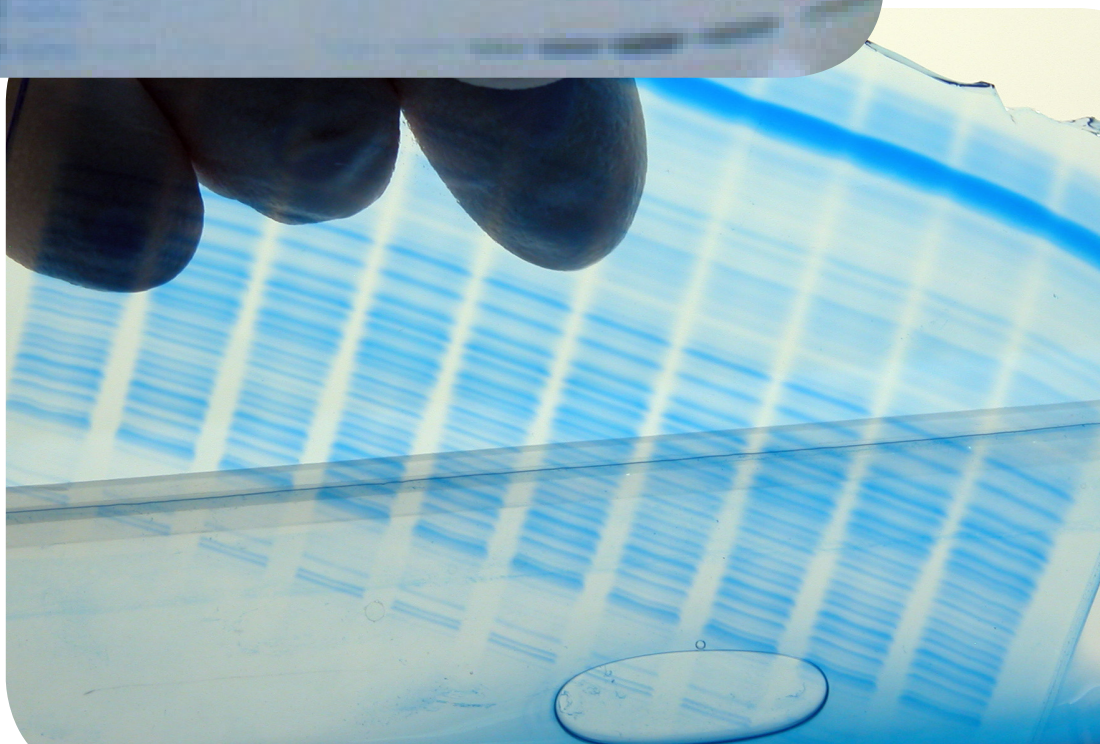


Protein Stains Handbook





BIOSCIENCES®

Protein Stains

Which Stain Is Most Suitable For Your Needs?

A major tool in the analysis of a species' proteome is the ability to separate and visualize the proteins by electrophoresis, 1D or 2D, and then the subsequent visualization of the proteins. Many protein stains are available for the analysis of electrophoresized proteins and this application note is an aid to selecting the correct stain. An essential consideration in selecting the appropriate stain is to establish the nature of the downstream application, as some stains are not compatible or interfere with some downstream processing applications. Other points researchers should consider in all their investigations is simplicity, ease of use and time. A final consideration is to decide what exactly you want to visualize; for example the range of protein species in your samples, or evaluate purification procedures, or monitor the isolation of protein molecules for its identification and determination of its nature (phosphoprotein/glycoprotein).

The choice of a staining method should be simple and fast. The method of choice should permit the visualization of protein species in less than 60-90 minutes without too many steps or complexity. A further important consideration is the question of sensitivity; ideally the staining method must be able to detect protein at the nanogram level, thus enhancing the chance of detecting less abundant proteins in any sample.

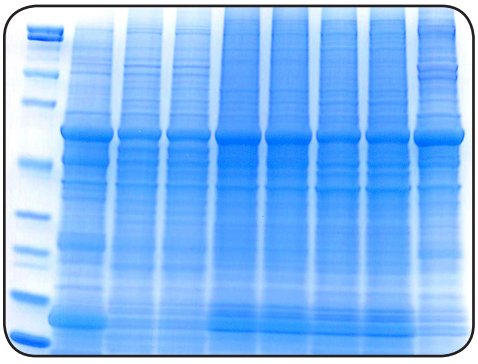
Below is a brief summary of protein stains, detailing their advantages and disadvantages.

COOMASSIE BLUE STAINING

The most common used protein stain is Coomassie Blue staining, which is based on the binding of Coomassie Brilliant Blue, which binds non-specifically to virtually all proteins. Coomassie Blue staining is approximately 50-fold less sensitive than silver staining, however due to its simplicity binding Coomassie Blue is preferred.

A final consideration in the choice of staining agent is the ability to remove the stain (destain). Staining methods that allow destaining of gels makes them more appealing, as destaining may be needed for certain downstream applications. In addition, staining may be too intense in some cases, and partial destaining may be required to visualize closely migrating proteins.

G-Biosciences offers several protein stains and destains that are an improvement on the "classical" staining methods.



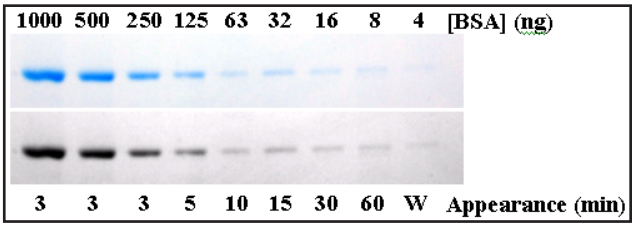
RAPIDstain™

RAPID*stain*™ is an ultra-sensitive and rapid stain based on Coomassie Blue dye. RAPID*stain*™ has a major advantage over conventional Coomassie Blue staining as it eliminates the lengthy fixation step. A further time saving factor of RAPID*stain*™ is that no mixing or preparation is required, simply pour RAPID*stain*™ onto gels and protein bands develops within 5-10 minutes. Complete staining achieved in 1 hour. RAPID*stain*™ only stains proteins, leaving a crystal clear background producing high band visibility. RAPID*stain*™ has the sensitivity to detect as little as 4-8ng BSA.

LabSafe GEL Blue™

LabSafe GEL Blue™ is an enhanced protein stain that is based on Coomassie dye that offers unsurpassed sensitivity and rapid band visualization. *LabSafe* GEL Blue™ is supplied in a ready-to-use format, which is added directly to protein gels following electrophoresis after a brief wash step.

The figure to the right depicts the sensitivity and the speed of band visualization. Briefly, serial dilutions (4ng-1µg) of bovine serum albumin (BSA) were reduced and loaded onto a SDS polyacrylamide gel. Following electrophoresis the gel was washed 3 x 5 minutes in deionized water to remove excess detergent. Enough *LabSafe* GEL Blue™ was added to cover the gel and was gently shaken for 1 hour. Protein bands (>250ng) appeared as earlier as 3 minutes and all bands >8ng were visible after 60 minutes of staining (see figure). The gel was washed (W) 2-3 times in deionized water to reveal the 4-8ng protein bands.



The result shows that *LabSafe* GEL Blue™ can detect protein levels as low as 4ng BSA. 60-1000ng BSA is detectable in 5-10 minutes and the low levels of BSA (4-8ng) become clearly visible when washed in water.

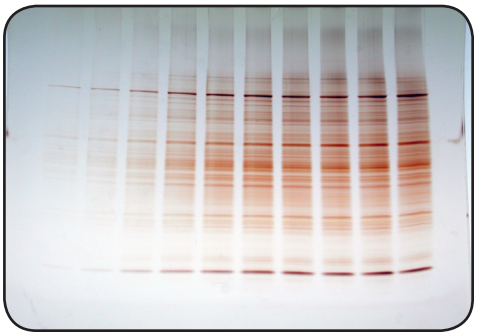
The image is also shown in black and white for better resolution of the 4 and 8ng lanes.

SILVER STAINING

Silver staining is a very popular method for protein detection, allowing for the detection of nanogram quantities of proteins, however silver staining has several drawbacks. Many silver staining methods use glutaraldehyde as a sensitizer. The use of which has two drawbacks; firstly, glutaraldehyde acts as a protein crosslinker making subsequent protein elution more difficult and, secondly, it modifies lysine residues preventing complete digestion and recovery of peptides. Silver ions also inhibit trypsin digestion by binding at the active site of trypsin and chymotrypsin, thus preventing peptide fragmentation for mass spectroscopy analysis. A final important consideration on the silver staining methods is the fact that they are unable to detect all proteins, particularly glycoproteins and proteins with large modified groups attached to their side-chains.

FASTsilver™

FAST*silver*™ is a nanogram sensitive silver staining kit that has been adapted by G-Biosciences to allow for the sensitive silver staining of proteins and nucleic acids, without some of the drawbacks of conventional silver stains. FAST*silver*™ kit does not utilize the protein modifier glutaraldehyde and therefore allows for the complete recovery of proteins and trypsin digested peptides. Our unique formulation leaves backgrounds clear, producing sharp images of proteins and nucleic acids and is able to detect as little as 1ng proteins and 0.3ng nucleic acids.

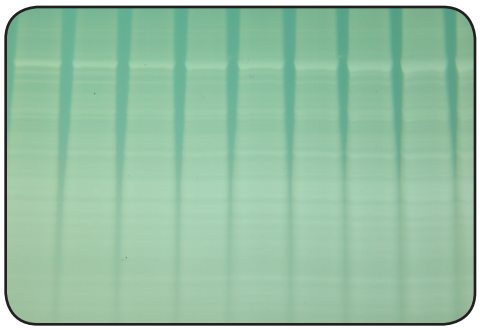


FOCUS™ *FASTsilver*™

FOCUS™ FAST*silver*™ is based on our popular FAST*silver*™ kit, but has been enhanced for use with 2D electrophoresis gels and for downstream mass spectroscopy analysis. FOCUS™ FAST*silver*™ is a nanogram sensitive silver staining kit that produces a crystal clear background and allows for maximal peptide recovery needed for analysis by mass spectrometry. FOCUS™ FAST*silver*™ has no glutaraldehyde step and therefore allows for complete protein and peptide recovery.

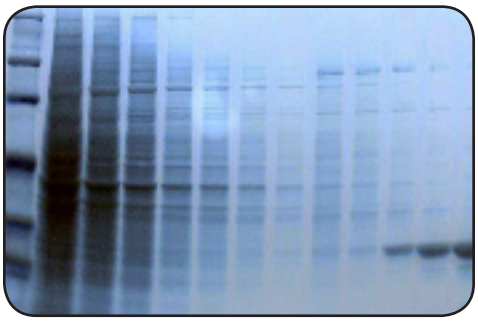
The FOCUS™ FAST*silver*™ kits are also supplied with *Silver*OUT™, G-Biosciences' own silver ion removing reagent, which enables the complete trypsin digestion and extraction of peptides needed for maximal recovery.

FOCUS™ FAST*silver*™ also detects as little as 1ng protein and 0.3ng nucleic acid.



Reversible Copper Stain™

Reversible Copper Stain™ is a single step stain for rapid detection of proteins resolved on SDS-PAGE. This kit is based on the principal of copper ions reacting with the SDS in the gel matrix leading to the deposition of a copper metal precipitate in the matrix. SDS-protein complexes in the matrix prevent the precipitation of copper ions producing a negative gel image consisting of clear protein bands visualized. Advantages of the *Reversible Copper Stain*™ is that no destaining is necessary, protein bands are visualized in as little as 5 minutes and the stain does not interfere with the electroelution of proteins or alter their biological properties. *Reversible Copper Stain*™ has a sensitivity of 0.1-0.5ng protein.

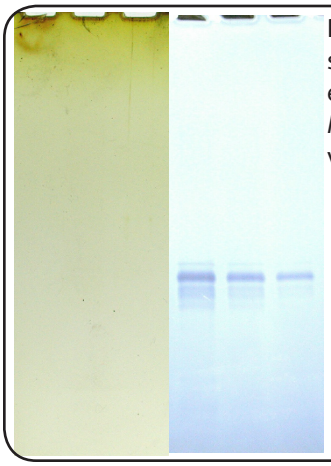


Reversible Zinc Stain™

Reversible Zinc Stain™ works by depositing a zinc metal precipitate in the SDS-gel matrix, which turns the gel opaque white, while the SDS coating on the proteins prevents the zinc stain from binding to the proteins, resulting in a negative image of the gel. Protein bands are visualized in as little as 10-15 minutes. Gels stained with the *Reversible Zinc Stain*™ can be destained 5 minutes before transfer or electroelution of proteins. This stain works with native, SDS denatured gels and gels containing glycine, tricine and a variety of primary-amine containing buffers. The sensitivity of *Reversible Zinc Stain*™ is 0.1-0.5ng protein and does not interfere with electroelution of proteins or alter their biological properties.

A major advantage of the negative stains is that they are able to detect proteins that are undetectable by silver stain, such as some glycoproteins and phosphoproteins.

If you fail to detect your proteins of interest using conventional staining methods, try G-Biosciences' 5 Minute *Reversible Copper Stain*™ or *Reversible Zinc Stain*™. They may develop protein bands previously undetected by other stains.



Phosvitin, a phosphoglycoprotein, was separated by SDS-PAGE and developed either by a silver staining method or the *Reversible Zinc Stain*™. Phosvitin was only visualized with the *Reversible Zinc Stain*™.

← Phosvitin

G-Biosciences has produced a protein stain designed for the detection of glycoproteins with the sensitivity of silver staining, *Glycoprotein Staining Kit*.

Glycoprotein Staining Kit™

Glycoprotein Staining Kit™ allows staining of glycoprotein bands that may not be visible if stained with ordinary Coomassie dye or silver staining methods. *Glycoprotein Staining Kit*™ kits are a silver enhancement of traditional glycoprotein staining methods. The kit combines the staining of glycoproteins with a dye, *GlycoBlue*, with G-Biosciences' FAST*silver*™, which results in at least a two fold increase in sensitivity for detection of proteoglycans and glycoproteins. *Glycoprotein Staining Kit*™ allows for the detection of both glycosylated and non-glycosylated proteins and has nanogram sensitivity.

Silver D-Stain™

Silver D-Stain™ allows the destaining and re-staining of gels stained with FAST*silver*™. Silver D-Stain™ can be used to reduce the staining of silver stained gels, allowing the visualization of proteins hidden by strongly stained protein or to completely de-stain gels and re-stain your gels or follow other downstream applications, such as Western transfer or sequencing. It is suitable for both protein and nucleic acid gels.

CAT. #	DESCRIPTION/ SIZE
786-31	RAPID*stain*™/ 1L
786-31G	RAPID*stain*™/ 1gal
786-35	*LabSafe* GEL Blue™/ 1L
786-35G	*LabSafe* GEL Blue™/ 1gal
786-30	FAST*silver*™/ 25 mini gels
786-240	FOCUS™ FAST*silver*™/ 25 mini gels
786-32CU	*Reversible Copper Stain*™/ 25 mini gels
786-32ZN	*Reversible Zinc Stain*™/ 25 mini gels
786-254	*Glycoprotein Staining Kit*™/ 25 mini gels
786-199	Silver™ D-Stain/ 25 mini gels
786-495	Coomassie Brilliant Blue R-250 Dye/ 10g
786-496	Coomassie Brilliant Blue G-250 Dye/ 10g
786-497	Coomassie Brilliant Blue G-250 Solution/ 1L
786-498	Coomassie Brilliant Blue R-250 Solution/ 1L

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