Removal of Detergents, Salts, Chaotropes and Reducing Agents with PAGE-Perfect™ Substantially Improves Protein Electrophoresis Resolution

Protein electrophoresis is routinely used in proteomic research, however many buffers and reagents used in preparation of protein samples have a deleterious effect on electrophoretic resolution. Commonly used interfering agents include detergents, chaotropes, salts and reducing agents. The presence of these contaminants, or interfering agents, results in band distortion and poor protein resolution. As a result, SDS-PAGE gels are hard to analyze and lack reproducibility.

PAGE-Perfect™ is a fast, efficient, two-step method for concentrating, cleaning and preparing samples for electrophoretic analysis resulting in reproducible, publication quality gels. Compatible with all premade and laboratory poured gel systems.

This application note compares electrophoretic resolution of proteins in various buffers containing common interfering agents before and after treatment with PAGE-Perfect™.

AIM

To evaluate PAGE-*Perfect*™ in the sample preparation of mouse liver lysates containing common interfering agents for SDS-PAGE analysis. Common interfering agents include chaotropes (8M urea), detergents (20% SDS, 10% Triton® X-100, 10% CHAPS), salts (1M Sodium chloride, 2.5M Ammonium sulfate) and sugars (40% sucrose).

METHOD

Concentrated samples of mouse liver lysate in 0.1M Tris (pH7.5) were diluted ~17-fold in the following buffers:

1. 0.1M Tris.HCl (pH7.5)
2. 1M Sodium chloride
3. 2.5M Ammonium sulfate
4. 20% SDS
5. 10% Triton® X-100
6. 8M Urea
7. 10% CHAPS
8. 40% Sucrose

10µg each sample was treated with the PAGE-*Perfect*™ kit. UPPA™-I was added to the sample, which was then placed on ice for 15 minutes, after which UPPA™-II was added. The samples were centrifuged to separate the precipitated protein from the soluble interfering agents. The precipitated proteins were extensively washed in PAGE-Wash, deionized water and then our proprietary OrgoSol buffer to remove all residual interfering agents.

Finally, the proteins are resuspended in PAGE-*Perfect*™ buffer and PAGE-Sample buffer. The entire sample was loaded on a 4-20% SDS-PAGE gel. In addition, 10µg untreated sample was loaded on a 4-20% SDS-PAGE gel. Both gels were silver stained with G-Biosciences FAST*silver*™ protein stain (Cat. # 786-30).

RESULTS & DISCUSSION

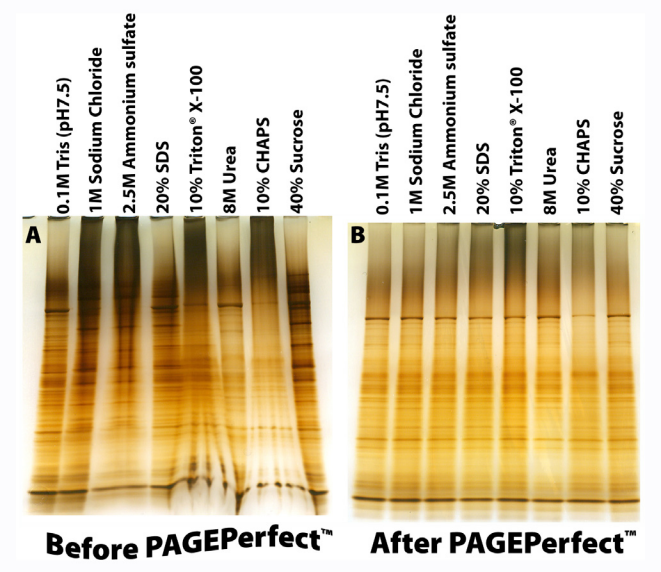

Figure 1: Analysis of mouse liver lysates before and after treatment with PAGE-Perfect™. A. 10mg untreated mouse liver lysates, in the presence of various interfering agents, were loaded onto 4-20% SDS-polyacrylamide gels. B. 10µg mouse liver lysates, in the presence of various interfering agents, were treated with PAGE-*Perfect*™ and then loaded onto 4-20% SDS-polyacrylamide gels. Both gels were stained with G-Biosciences FAST*silver*™ stain.

Figure 1A clearly demonstrates the effects of common laboratory agents on protein electrophoresis. All the reagents had an effect on protein electrophoresis, with the detergents (Triton® X-100 and CHAPS) and ammonium sulfate being the most detrimental. In addition, some chaotropes, such as guanidine·HCl, have a detrimental effect prior to electrophoresis as they cause protein precipitation when mixed with sample loading buffers.

The use of PAGE-*Perfect*™ (Figure 1B) removes the interfering agents from the proteins and allows the samples to be reproducibly resolved on SDS polyacrylamide gels, eliminating all the artifacts seen in figure 1A. Treatment with PAGE-*Perfect*™ results in higher band visibility, greater protein resolution, and no band distortion.

PAGE-*Perfect*™ eliminates the electrophoretic artifacts, concentrates dilute (1mg/ml) protein samples and is suitable for 1-100µg protein. It allows for the reproducible generation of high quality, publication quality polyacrylamide gels.

PAGE-*Perfect*™ is ideal for the removal of most salts, chaotropes, detergents and sugars. It is an ideal sample preparation step following protein precipitation with ammonium sulfate, lysis of samples in urea and thiourea, solubilization of inclusion bodies with guanidine·HCl and membranes solubilized with various detergents

ORDERING INFORMATION

Cat. #	Description/ Size
786-123	PAGE-*Perfect*™/ 100 preps
786-123T	PAGE-*Perfect*™/ 5 preps
786-30	FAST*silver*™/ 25 mini gels

REFERENCES

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