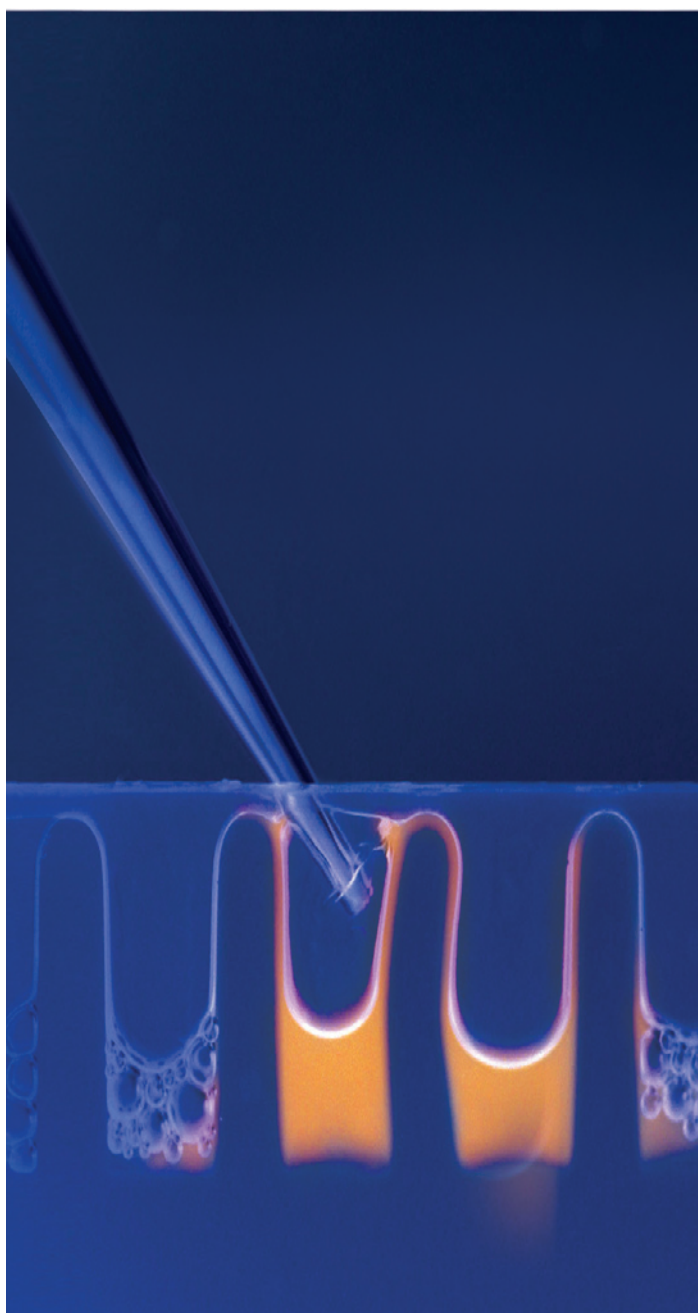


## White Paper:

# Optimized Products for Improved Results in SDS-PAGE and Western Blotting



By Hugh White, Lonza Rockland, Inc. and Leon de Bruin, Lonza Verviers SPRL

### Introduction

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Separation of proteins by SDS-PAGE and subsequent immunodetection (Western Blotting) are key applications in the discovery, identification and analysis of proteins. Lonza offers optimized gel matrices and reagents to significantly improve the quality and consistency of results in these critical techniques.

In this article we demonstrate the protective and verification functions of new ProSieve® ProTrack™ Dual Color Loading Buffer; the advantages of ProSieve® Color and Unstained Protein Markers for sizing and monitoring; the quality of separation of proteins on PAGER® Precast Gels; and the improved staining properties of new ProSieve® Blue Protein Staining Solution.

### Materials and Methods

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#### Preparation of protein samples and markers

We compared the separation patterns of three different proteins on PAGER® Gels (Lonza). Protein samples were prepared from 10 mg/ml stocks of phosphorylase B, BSA, and myoglobin using ProSieve® ProTrack™ Dual Color Loading Buffer (Lonza 00193861), or other loading buffer (Bio-Rad). Samples were denatured by incubation at 95°C for 15 minutes, before being loaded on the gel (Fig. 1). In a parallel experiment, a dilution series of tubulin protein samples were detected following Western Blotting (Fig. 4).

We used 10 µl/lane of the ProSieve® Color Protein Marker, 10 – 190 kDa (Lonza 50550) and 10 µl/lane of the ProSieve® QuadColor™ Protein Marker, 4.6 – 300 kDa (Lonza 00193837) (Figs. 2, 3 & 5). Unstained protein markers were used at 5 µl/lane for both the ProSieve® Unstained Protein Marker, 5 – 225 kDa (Lonza 50547) and the ProSieve® Unstained Protein Marker II, 10 – 200 kDa (Lonza 00193839) (Fig. 6).

#### Separation of proteins

Protein samples and markers were separated on 10 x 10 cm, 4 – 20% PAGER® Gels (Lonza 59517), run at 125 – 200 Volts for

60 – 90 minutes, in a Lonza PAGER® Minigel Chamber (Lonza 59905), using a 1X AccuGENE® Tris-Glycine SDS buffer (Lonza 50880).

#### Detection of proteins on a gel

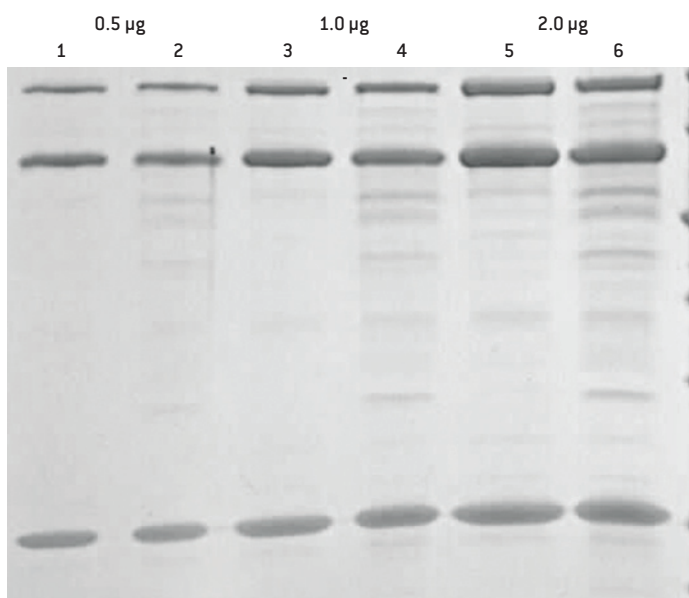
Unstained protein markers and protein samples were stained using ProSieve® Blue Protein Staining Solution (Lonza 00193862), and compared with standard Coomassie Brilliant Blue staining (Fig. 7).

#### Transfer of proteins to a blot

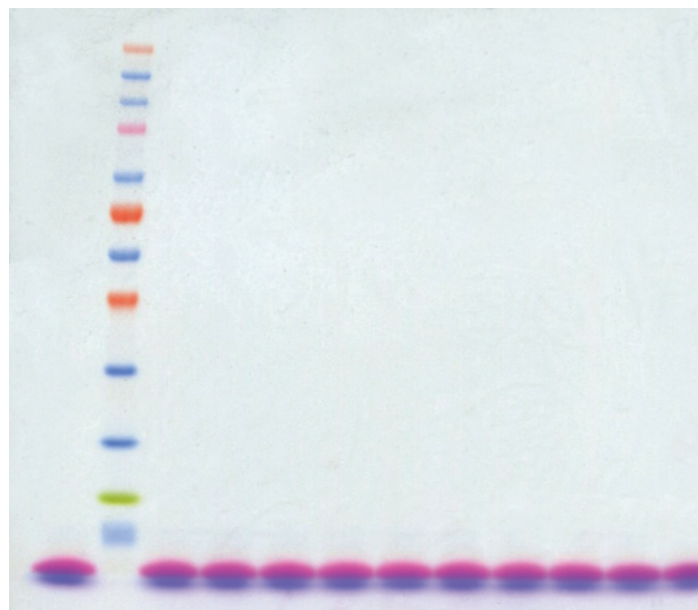
Gels were blotted on a membrane (Protran BA85-SD nitrocellulose) by semi-dry transfer (25 V at 400 mA for 60 min) using standard Towbin transfer buffer (1X Tris Glycine with 20% methanol added). To verify the complete transfer of the proteins on to the blot, we used both the pink dye component of the ProSieve® ProTrack™ Dual Color Loading Buffer (Lonza 00193861), and the ProSieve® Color Protein Markers. (Fig. 3).

The  $\alpha$ -tubulin samples were detected using  $\alpha$ -tubulin specific monoclonal antibody (Accurate Scientific) and a traditional HRP tagged secondary antibody, or using the One-Step Western Advanced Kit, Mouse (GenScript L00242). SuperSignal West Pico reagents were used for the ECL detection. Bands were excited using a Dark Reader® transilluminator and imaged (Fig. 4)

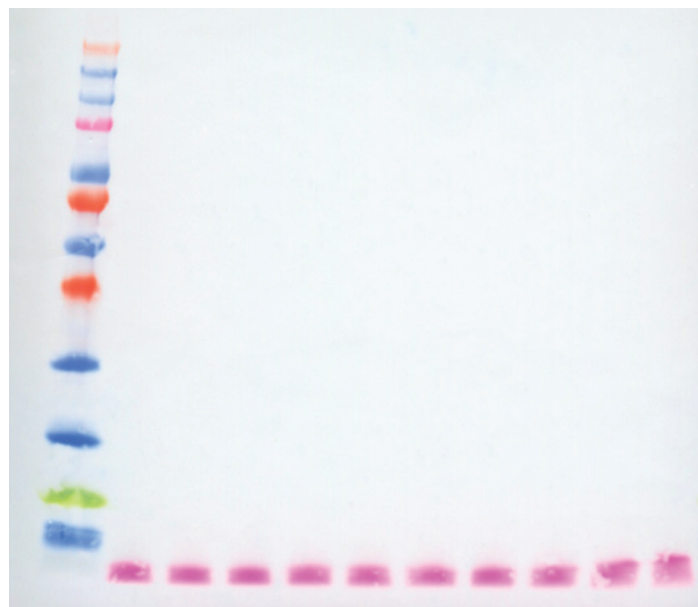
## Results



**Fig. 1. Protection of protein samples using ProSieve® ProTrack Dual Color Loading Buffer.** Dilutions of Phosphorylase B, BSA, and myoglobin proteins, separated on a 4 – 20% PAGER® Gold Gel. Lanes 1, 3 and 5 are protected by ProSieve® ProTrack™ Dual Color Loading Buffer, Lanes 2, 4 and 6 are proteins prepared and run in a standard loading buffer. Notice the amount of degraded proteins in the non-protected lanes.

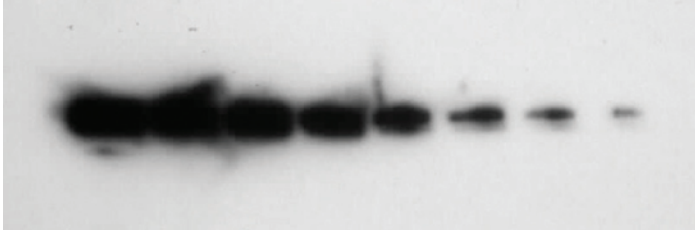


**Fig. 2. Monitoring separation with ProSieve® Color Protein Marker and ProSieve® ProTrack™ Dual Color Loading Dye.** Dilution series of tubulin run on a 4 – 20% PAGER® Gold gel. The ProSieve® QuadColor™ Protein Marker in the second lane provided visual identification of run distance on the gel. ProSieve® ProTrack™ Dual Color Loading Buffer in the other lanes indicated the running front of the proteins (blue dye), and verification of the subsequent transfer efficiency of the proteins to the blot (pink dye).

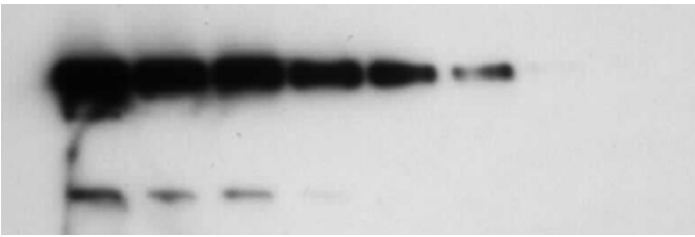


**Fig. 3. Confirmation of protein transfer with ProSieve® QuadColor™ Protein Marker and ProSieve® ProTrack Dual Color Loading Buffer.** Dilution series of tubulin separated on a 4 – 20% PAGER® Gold gel, and then blotted onto a membrane. Bands from the ProSieve® QuadColor Protein Marker and the pink dye from the ProSieve® ProTrack™ Dual Color Loading Buffer transferred to the membrane, providing clear indication of protein transfer efficiency prior to membrane staining.

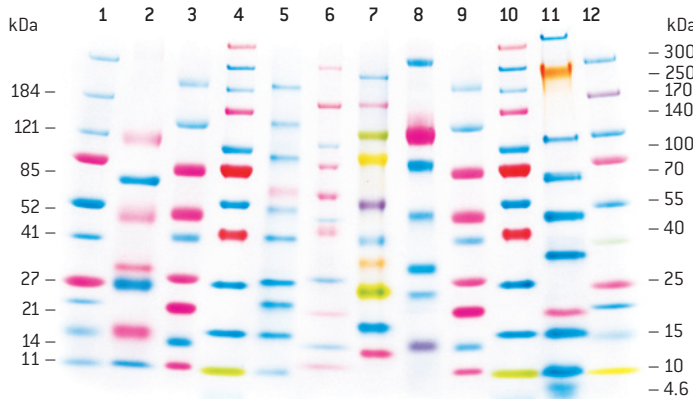
**A) ProSieve® ProTrack™ Dual Color Loading Buffer**



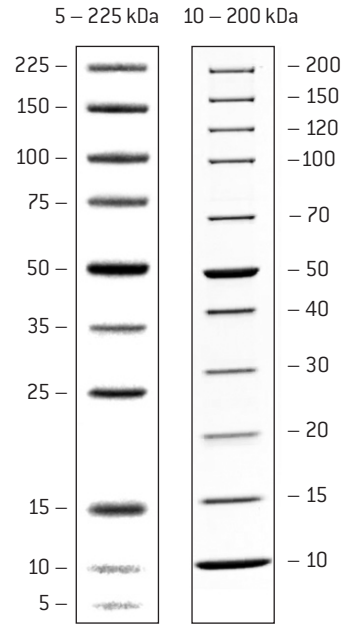
**B) Standard Loading Buffer**



**Fig. 4. Western detection of protein samples prepared with ProSieve® ProTrack™ Dual Color Loading Buffer.** Detection of tubulin dilution series following blotting; Gel A used samples prepared with ProSieve® ProTrack™ Dual Color Loading Buffer, while Gel B used samples prepared with standard loading buffer. Note the higher sensitivity of transfer and no evidence of degradation (lower MW bands) with the samples prepared in ProSieve® ProTrack™ Dual Color Loading Buffer.

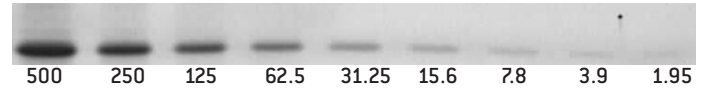


**Fig. 5. Comparison ProSieve® Color Protein Markers with other popular color markers.** Protein color markers from various suppliers run on a 10 x 10 cm 4 – 20% PAGEr® Gel. Lanes 3, 4, 9 and 10 contain Lonza markers. Lane 1 Bio-Rad® Precision Plus Dual Color Standard; Lane 2 Sigma ColorBurst™ Electrophoresis Marker; Lane 3: Lonza ProSieve® Color Protein Marker; Lane 4 Lonza ProSieve® QuadColor™ Protein Marker; Lane 5 Invitrogen BenchMark™ Pre-Stained Ladder; Lane 6 Invitrogen Novex® Sharp Pre-Stained Standard; Lane 7 GE Full Range Rainbow™ Marker; Lane 8 Pierce 3-Color Pre-Stained Marker; Lane 9 Lonza ProSieve® Color Protein Marker; Lane 10 Lonza ProSieve® QuadColor Protein Marker; Lane 11 Invitrogen SeeBlue® Plus 2 Pre-Stained Standard; Lane 12 Bio-Rad® Precision Plus Kaleidoscope™ Standard. Note the sharpness of the bands and low background with the Lonza markers vs. some other color markers.

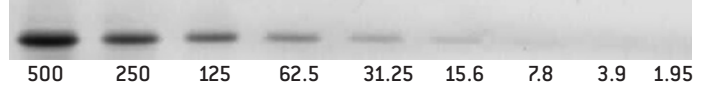


**Fig. 6. ProSieve® Unstained Protein Markers.** Unstained markers run on a 10 x 10 cm, 4 – 20% PAGEr® Gel, and stained with ProSieve® Blue Protein Staining Solution. Lane 1 ProSieve® Unstained Protein Marker, 5 – 225 kDa; Lane 2 ProSieve® Unstained Protein Marker II, 10 – 200 kDa.

**A) ProSieve® Blue Protein Staining Solution**



**B) Coomassie Blue**



**Fig. 7. Gel staining using ProSieve® Blue Gel Stain.** Dilution series of a 15 kDa band separated on a 4 – 20% PAGEr® Gel and then stained with ProSieve® Blue Protein Staining Solution (Lonza, Gel A) or Coomassie Blue (Gel B).

**Conclusion**

Lonza offers an optimized set of products for protein separation and Western Blotting that, when used together, significantly improve protein integrity, resolution quality and Western transfer efficiency.

New ProSieve® ProTrack™ Dual Color Loading Buffer protects proteins during the sample preparation phase, and during SDS-PAGE, by stabilizing pH fluctuations caused by temperature differences, that can lead to degradation of proteins (Fig. 1). Use of ProTrack™ not only protects protein integrity in SDS-PAGE, but also improves the level of transfer in Western Blotting (Fig. 4).

The two dyes incorporated in this buffer function as run front indicator (Fig. 2), and as verification of transfer efficiency (Fig. 3).

ProSieve® Unstained Protein Markers exhibit sharp bands for accurate sizing of unknown protein bands over a broad size range. ProSieve® Color Protein markers provide sharp, colorful visual positioning of proteins during and after SDS-PAGE (Fig. 2) and provide accurate confirmation of Western transfer (Fig. 3).

New ProSieve® Blue Protein Staining Solution is approximately 10-times more sensitive than a standard Coomassie stain (detecting down to 2 – 4 ng of protein). Quantitative image analysis shows a linearity of staining with ProSieve® Blue Protein Staining Solution in the range shown (1 – 500 ng). Further, ProSieve® Blue Stain is fast and simple to use, does not require any destaining, and overstaining is not possible.

Finally, PAGER® Gels provide the ideal matrix for protein separation, yielding consistently sharp bands, fine resolution, and efficient transfer of proteins up to 250 kDa. PAGER® cassettes are easy-to-load (lane markers and tinted color), easy-to-open (comb serves as the tool), and have the longest guaranteed shelf life of any other Tris-Glycine gel on the market (>10 weeks). The gels are available in two sizes to fit popular chambers.

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