Imaging Criterion™ TGX Stain-Free™ gels compared to standard Coomassie staining procedures using G:BOX imaging system

Introduction

The ability to be able to visualise gels without a staining step has proved very popular. Stain-free technology has many advantages over staining procedures such as Coomassie blue.

One major advantage of the stain-free technology is that it eliminates the need for a lengthy de-staining step making visualisation faster and more efficient.

There are several different stain-free technologies currently available for this application note the focus will be on Criterion stain-free gels.

Stain-free gels contain a special Trihalo compound that covalently binds to proteins' tryptophan residues when activated with UV light. The activation of the Trihalo compounds in the gels adds a 58Da adduct to available tryptophan residues and is required for protein visualisation.

Proteins which do not contain tryptophan residues will not be detected.

Materials and methods

Samples

Purified proteins used in this experiment were BSA (0.3% Tryptophan content), Carbonic anhydrase (2.3% Tryptophan) and Lysozyme (3.4% Tryptophan) were purchased from Sigma-aldrich, UK.

Electrophoresis

Electrophoresis was performed using Criterion TGX Stain-Free gel 4-20% (BIO-RAD, UK). Samples were prepared in Laemmli sample buffer and heated at 70^{0}C for 10 minutes. ColorBurst™ Electrophoresis markers were loaded as provided.

Gels were run using Criterion electrophoresis cell at 200V until the dye front reached the bottom of the gel. Gel was run using Tris, Glycine, SDS buffer (0.25M Tris, 1.92M Glycine and 1% SDS).

Imaging

Criterion TGX Stain-Free gel was imaged using a G:BOX Chemi XR5 system. The gel was exposed to 5 minutes of mid wave UV (302nm) from a UV transilluminator and a UV filter was placed in front of the camera. After a 5 minute exposure, an image of the gel was captured using a UV transilluminator and filtUV. An exposure time of ~2 seconds was required.

The Criterion TGX Stain-Free gel was additionally stained with ProtoBlue Safe (GeneFlow, UK) and imaged with G:BOX Chemi XR5.

Results

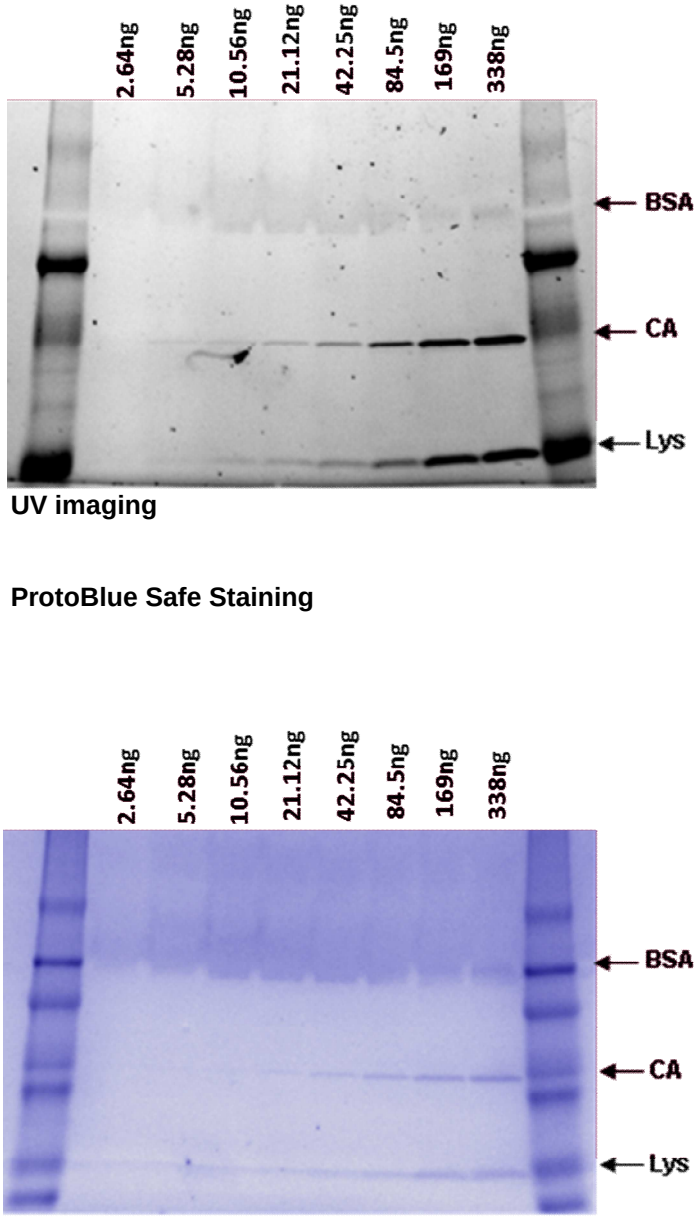


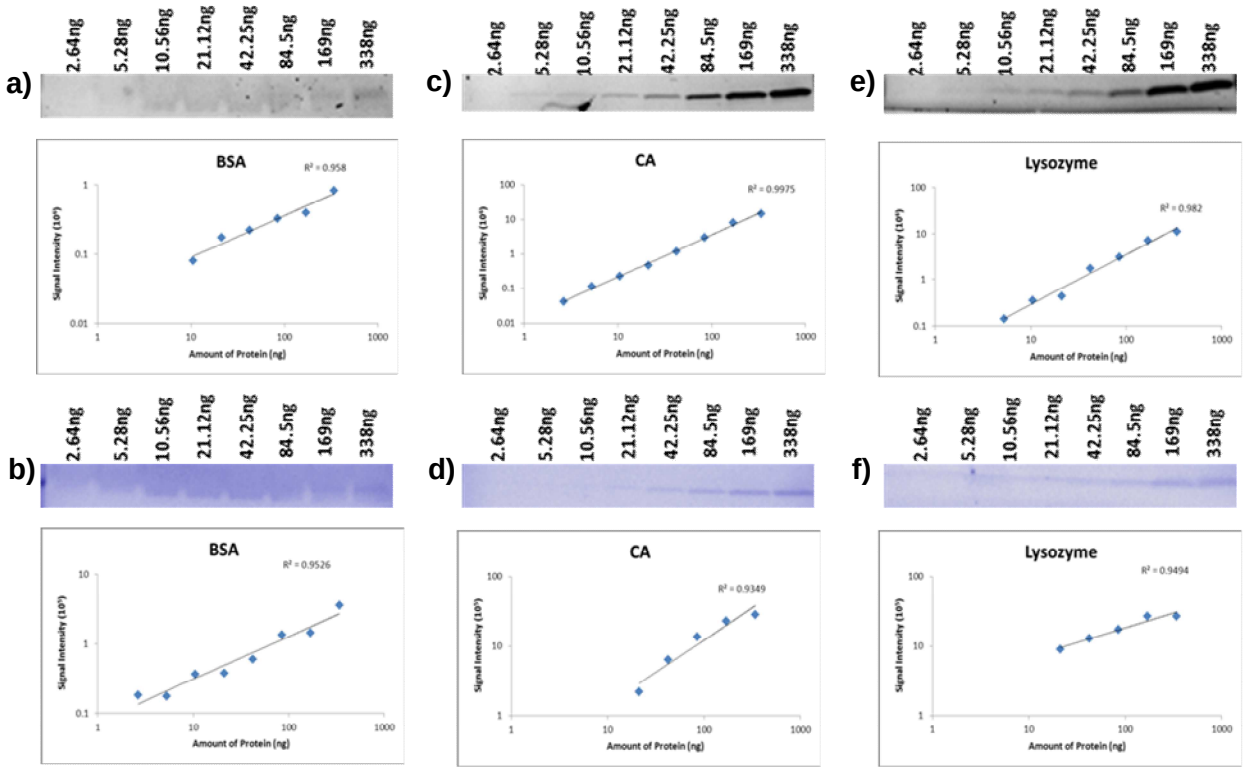
Figure 1- Stain-free gel imaging compared to ProtoBlue safe staining.
Serial dilutions (338-2.64ng) of a protein mixture (BSA, Carbonic anhydrase and Lysozyme) were run on a Criterion 4-20% TGX Stain-Free gel and imaged with UV on a G:BOX Chemi XR5 system and additionally stained with ProtoBlue Safe stain.

Determining Linear range and Limit of Detection (LOD)

A mixture of proteins including BSA, CA and Lysozyme were prepared in a 2-fold serial dilution ranging from 338-2.64ng then loaded onto a Criterion TGX Stain-Free gel. This mixture of proteins was chosen due to its varied percentage content of tryptophan.

The amount of BSA, Carbonic anhydrase and Lysozyme loaded on to the gel was plotted against raw volume (Signal Intensity) determined by GeneTools analysis software (Syngene, UK) (**Figure 2**).

The linearity and sensitivity of the stain free method is comparable to that of more traditional staining techniques such as Coomassie Blue Safe (ProtoBlue Safe) and for some proteins (CA and Lysozyme) showed greater level of sensitivity (**Figure 2**). BSA due to its low level content of tryptophan showed lower sensitivity compared to ProtoBlue Safe staining.



	Protein	Limit of Detection (LOD) (ng)	Linearity (R^2)
Stain Free	BSA	10.56	0.958
	CA	2.64	0.9975
	Lysozyme	5.28	0.982
ProtoBlue Safe	BSA	2.64	0.9526
	CA	21.12	0.9349
	Lysozyme	21.12	0.9494

Table I

Figure 2- Determination of linear range and limit of detection (LOD) of BSA, CA and Lysozyme with stain free and ProtoBlue Safe techniques

Proteins BSA, CA and Lysozyme diluted by 2-fold dilution starting at 338ng. Criterion TGX Stain-Free gel was either exposed to UV light **a)** BSA, **c)** CA and **e)** Lysozyme or stained with ProtoBlue safe **b)** BSA, **d)** CA and **f)** Lysozyme all imaged using G:BOX Chemi XR5 system.

Signal Intensity was calculated from raw volume using GeneTools software. Limit of detection and R^2 values are shown in **Table I**.

Conclusions

By using the recommended lighting and filter combination, 302nm UV transilluminator and UV filter, users can successfully image Criterion TGX Stain-Free gels using any Syngene G:BOX imaging systems.

Stain-free method has comparable sensitivity to that of more traditional techniques such as, Coomassie Blue Safe staining and also has the potential to significantly reduce the lengthy SDS-PAGE workflow.

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