Superior electrophoresis results with Lonza reagents and the Azure cSeries imaging systems

Introduction

Lonza, a leader in electrophoresis reagents, and Azure Biosystems, manufacturer of gel doc and Western blot imaging systems, have teamed up to demonstrate how to easily obtain excellent DNA and protein gel electrophoresis data. Lonza provides a complete line of reagents for nucleotide and protein electrophoresis including precast gels, buffers, stains, and markers for fast and reliable electrophoresis. Using precast gels saves time and allows for reproducible results due to tight quality control. The cSeries family of digital imaging systems from Azure Biosystems offers a range of instruments to suit imaging needs from gel documentation to quantitative imaging of multicolor fluorescent Western blots. Each instrument is capable of imaging commonly used DNA and protein stains, having illumination sources that include both white light and dual-UV transilluminators, as well as epi white and blue light. The cSeries instruments include user-friendly interfaces to make capturing the best possible image a breeze.

This application note demonstrates the high-sensitivity and quality results that can be obtained combining the expertise of these two companies for DNA and protein electrophoresis and analysis.

Fast nucleotide electrophoresis and sensitive detection with the FlashGel™ System and cSeries imager

The FlashGel™ System from Lonza consists of precast agarose gel cassettes that are run in an accompanying dock. Electrophoresis does not require preparation of running buffer or a separate staining step. Each gel contains a proprietary stain that is 5 to 20 times more sensitive than ethidium bromide and that can be imaged using either blue light or UV light with the same settings and filters that would be used with ethidium bromide. The Azure Biosystems cSeries instruments offer multiple options for imaging the FlashGel™ dye; UV transillumination, UV transillumination with a blue conversion screen that essentially converts the light source to a blue transilluminator, and epi blue light.

Figure 1 shows the excellent separation, sensitivity, and image quality obtained imaging a double tier FlashGel™ with the cSeries, using the UV transilluminator and blue conversion screen. The image was captured using a 10 second exposure. Fragments ranging from 50 bp to 1500 bp in size were well resolved on a 2.2% agarose gel in less than 5 minutes. High reproducibility is seen across the gel and across both tiers and the image demonstrates uniform intensity across the gel with excellent signal-to-noise. For samples loaded and imaging specifics, please see experimental details at the end of the application note.

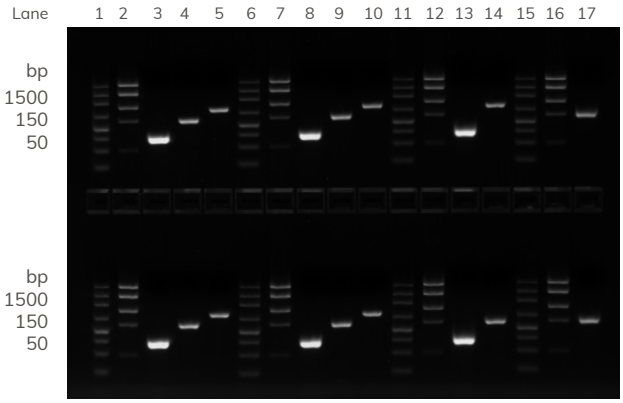

Figure 1. FlashGel™ imaged with the Azure cSeries imager, using UV transilluminator with blue conversion screen.

Figure 2 compares the limit of detection achieved when imaging a FlashGel™ System with the cSeries imaging system using either UV transillumination with a blue light conversion screen (Figure 2A), UV transillumination (Figure 2B), or epi blue light (Figure 2C). Though imaging with the blue conversion screen provides the optimal balance of sensitivity and low background, in every image a band containing less than 0.2 ng of nucleic acid

is easily detected. For details of samples loaded and imaging settings, please see experimental details at the end of the note.

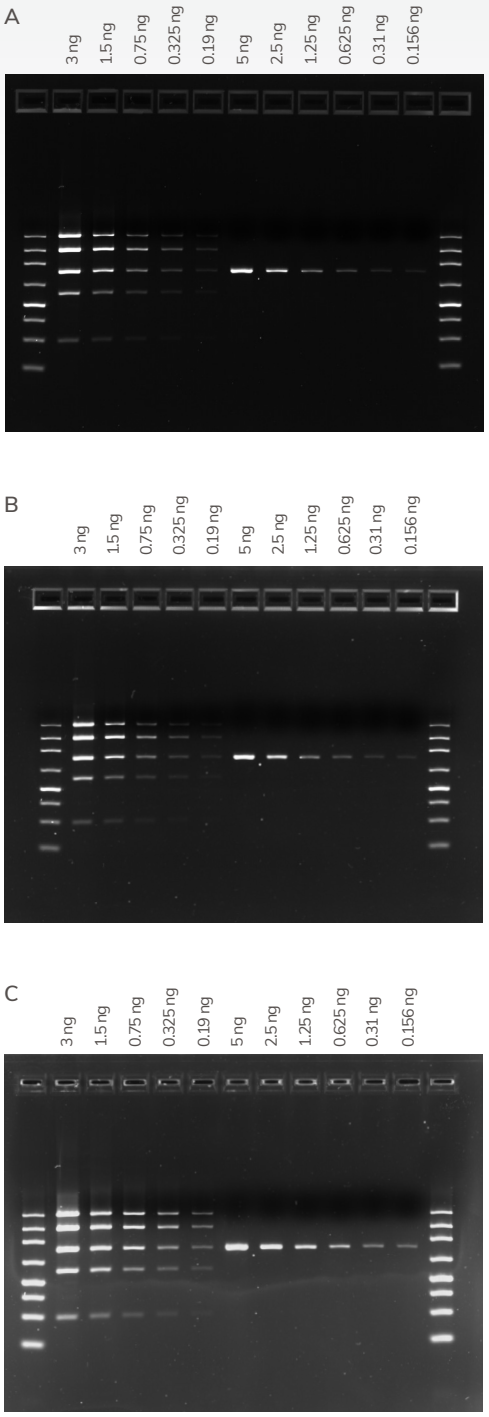

Figure 2. Comparison of sensitivity obtained imaging a FlashGel™ with the Azure cSeries imager using either (A) UV transilluminator with blue conversion screen, (B) UV transilluminator (UV 302), or (C) epi blue light.

Accurate electrophoresis and sensitive detection of larger-format Latitude™ gels with the cSeries imaging system

Latitude™ gels are precast agarose gels containing ethidium bromide. The gels are precision cast for high accuracy and reproducibility. Figure 3 shows a midi 1% SeaKem™ LE Plus gel imaged using a cSeries imager and UV transilluminator. The large imaging area of the cSeries instrument is well able to handle the larger-format gel (10x15 cm) and the even lighting and exposure reveal the highly reproducible electrophoresis of samples both across the entire gel and between the top and bottom set of wells. For details of samples loaded and imaging settings, please see experimental details at the end of the note.

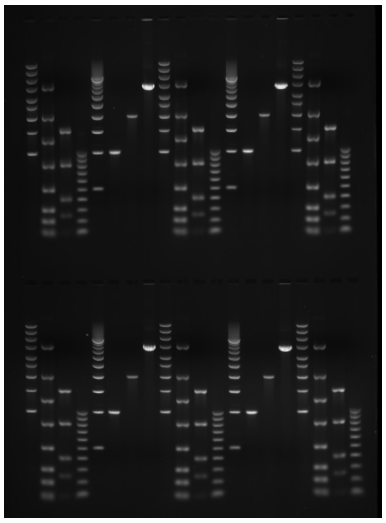

Figure 3. Imaging a Latitude™ Midigel with the Azure cSeries.

cSeries imaging of protein gels stained with ProSieve™ EX Safe Stain

For routine protein electrophoresis, PAGEr™ Gold precast Tris-glycine gels provide sharp resolution in a convenient, easy-to-use package. Figure 4 shows a PAGEr™ Gold gel stained with Lonza's ProSieve™ EX Safe Stain and imaged using white light transillumination on the Azure cSeries imager. The image demonstrates high sensitivity and excellent signal-to-noise. For details of samples loaded and imaging settings, please see experimental details at the end of the note.

Figure 5 demonstrates the limit of detection for a PAGEr™ Gold gel stained with ProSieve™ EX Safe Stain. A series of two-fold serial dilutions of carbonic anhydrase were loaded on the gel. A band containing less than 10 ng of protein is easily visualized in the image.

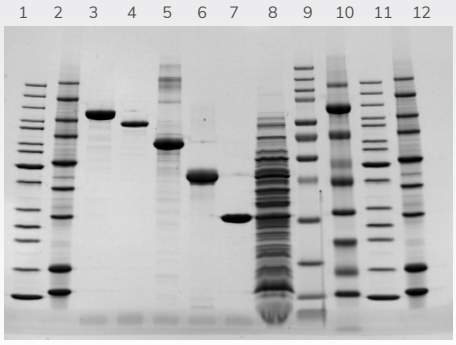

Figure 4. PAGEr™ Gold protein gel stained with ProSieve™ EX Safe Stain and imaged using the white light transilluminator of the Azure cSeries.

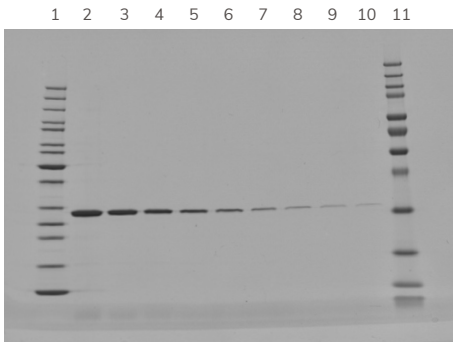

Figure 5. High sensitivity of ProSieve™ EX Safe Stain-stained gel imaged with the Azure cSeries.

Maximize convenience without sacrificing quality

The data presented demonstrate the high-quality results that result from combining the convenience of Lonza's precast gels and other reagents with the flexible, high-resolution imaging of the Azure cSeries imaging systems. The cSeries imagers provide light sources and filters compatible with all commonly used nucleotide and protein stains, including the proprietary stain included in Lonza's nucleotide FlashGel™ as well as Lonza's ProSieve™ EX Safe Stain for protein. The large imaging area of the cSeries instruments allows imaging of both small and large gels and the user-friendly interface makes image capture a breeze. To learn more about Lonza electrophoresis products please visit the Lonza website and to learn more about the cSeries imaging systems please visit www.azurebiosystems.com.

Experimental details

Figure 1

DNA samples were separated on a 2.2% 16+1 well double tier FlashGel™ DNA Cassette (Lonza product #57032). The samples loaded were as follows: lane 1, 4 µL 50-1500 bp FlashGel™ Marker (Lonza product #57033); lane 2, 4 µL FlashGel™ QuantLadder (Lonza product #50475); lane 3, 5 µL 150 bp DNA Fragment; lane 4, 5 µL 250 bp DNA fragment; lane 5, 5 µL 350 bp DNA fragment. The samples were then repeated across the gel. DNA fragments were diluted in 1X FlashGel™ Sample Buffer (Lonza). The gel was run for 4.5 minutes at 275 V. Imaging was conducted with a cSeries imager using the UV transilluminator at 302 nm with blue light conversion screen (Azure SKU AC1025). Exposure was 10 seconds.

Figure 2

DNA samples were separated on a 2.2% 12+1 well single tier FlashGel™ DNA Cassette (Lonza product #57031). The samples loaded were as follows: lane 1, 50-1500 bp DNA markers (Lonza product #57032); lanes 2-6, serial two-fold dilutions of FlashGel™ QuantLadder (Lonza product #50475) starting with 3 ng of the smallest (100 bp) band in lane 2; lanes 7-12, serial two-fold dilutions of a 400 bp DNA fragment starting with 5 ng in lane 7; lane 13, 50-1500 bp DNA marker. The gel was run for 8 minutes at 275 V. Imaging with UV transillumination and blue conversion screen (Azure SKU AC1025) was conducted using a 10 second exposure. Imaging with UV transillumination (302 nm) was conducted using the AutoExposure function of the cSeries. Imaging with epi-blue light was conducted with an exposure time of 2 seconds.

Figure 3

DNA samples were separated on a 1% SeaKem™ LE Plus gel containing ethidium bromide (Lonza product #57230). Samples loaded were as follows: lane 1, 4 µL 1-10 kb DNA Marker (Lonza product #50471); lane 2, 8 µL FlashGel™ 100-4000 bp DNA Marker (Lonza product #50473); lane 3, 8 µL FlashGel™ QuantLadder (Lonza product #50475); lane 4, SimplyLoad™ 100 bp DNA ladder (Lonza product #50327); lane 5, SimplyLoad™ 500 bp DNA ladder (Lonza product #50329); lane 6, 1 kb DNA fragment (BioVentures); lane 7, 2 kb DNA fragment (BioVentures); lane 8, 4 kb DNA fragment (BioVentures). The samples were then repeated across the gel. The gel was set up in an Owl B2 chamber with 1X TBE (with 0.5 µg/mL EtBr). The gel was run at 115 V (approximately 5 V/cm) for 85 minutes. The gel was imaged using UV transillumination at 302 nm. Exposure time was 6 seconds.

Figure 4

Protein samples were separated on a 4-20% PAGEr™ Gold gel (Lonza product #58105). Samples loaded were as follows: lane 1, 5 µL PageRuler Marker (Thermo Fisher); lane 2, 4 µL ProSieve™ Unstained Marker (Lonza); lane 3, 1 µg ß-galactosidase; lane 4, 2.5 µg Phosphorylase B; lane 5, 2 µg BSA; lane 6, 2 µg ovalbumin; lane 7, 2 µg carbonic anhydrase; lane 8, 4.5 µL E. coli LE 392 lysate; lane 9, 5 µL ProSieve™ QuadColor™ Protein Marker (Lonza product #193837); lane 10, 6.5 µL ProSieve™ Color Marker (Lonza); lane 11, 5 µL PageRuler Marker (Thermo Fisher); lane 12, 3 µL ProSieve™ Unstained Marker (Lonza). The gel was run for 40 minutes at 200 V. After electrophoresis, gel was placed immediately into ProSieve™ EX Safe Stain (Lonza product #00201455), heated in a microwave for 55 seconds, then stained with shaking for 20 minutes. The gel was then destained by pouring off the stain, adding water, and warming in a microwave with a piece of paper towel to absorb excess stain. The gel was imaged on the cSeries imager using the White Light Table (Azure SKU AC1029) and default settings for aperture and focal height.

Figure 5

Protein samples were separated on a 4-20% PAGEr™ Gold gel (Lonza product #58105). Samples loaded were as follows: lane 1, PageRuler Marker (Thermo Fisher); lanes 2-10, two-fold serial dilutions of carbonic anhydrase starting with 2 µg of protein in lane 2; lane 11, ProSieve™ QuadColor™ Protein Marker (Lonza product #193837). The gel was run for 40 minutes at 200 V. After electrophoresis, the gel was stained, destained, and imaged as described for Figure 4.

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