The FluorChem® Q: Sensitive and Rapid I-D Gel Imaging

Application Note 121

Abstract

The FluorChem Q is a versatile imaging system that is capable of capturing highly sensitive gel images in addition to detecting and quantifying multicolor Western blots. Here the FluorChem Q is shown to be a powerful tool in both DNA and protein imaging.

Introduction

Gel imaging is a critical component in a wide variety of today's life science applications, and the need for clear, precise, highly sensitive images is crucial. Cell Biosciences, a leading provider of digital documentation systems, introduces the FluorChem Q imaging system to provide users the flexibility to image both fluorescent and chemiluminescent Western blots with the same instrument and to provide quantitative accuracy necessary for rigorous data analysis. In addition to providing the tools for Western detection, the FluorChem Q also has the ability to image DNA and protein gels using the built-in dual wavelength UV trans-illuminator or the epi light sources at 475nm, 534nm and 632nm wavelengths. The ability to cover such a broad range of applications makes the FluorChem Q a very cost-effective multi-purpose scientific imager.

The purpose of this application note is to illustrate the gel imaging capabilities of the FluorChem Q. This study demonstrates that the FluorChem Q is capable of capturing highly sensitive gel images, in addition to detecting and quantifying multicolor Western blots.

Methods

DNA separation in agarose gel. A 2-fold serial dilution of a 1kB DNA ladder (Fisher BioReagents BP2578100) was loaded onto four separate agarose gels (1%) containing no dye. The initial DNA concentration was as supplied by the manufacturer, and subsequent dilutions were made with 1x loading dye. This marker contains 13 bands ranging in size from 300-10,000bp. 5µL was loaded per well. Using the mini-submerged system from Bio-Rad, gels were run at 40V for the first 20 minutes and at 75V for 80 minutes. The gels were then stained in 50 ml of staining solution. Four dyes were used: ethidium bromide, SYBR® Green I, SYBR® Gold, and SYBR® Safe.

Protein separation in polyacrylamide gel. The commercial pre-made polyacrylamide mini-gels used in these experiments contained 8-16% acrylamide Tris-HCl buffer. Gels were run in a standard Laemmli system at 30V for the first 30 minutes and then at 90V for 3-4 hours. A protein marker mixture (Invitrogen P6649) was used to prepare the samples. Two fold serial dilutions were made from a sample containing approximately 250ng/µL of each protein in the marker in the standard Laemmli loading buffer. Ten dilutions were prepared. 10µL of each sample was loaded per lane.

Protein gel staining was performed as suggested by the manufacturer for each protein gel stain. The volume of staining solution was 50ml for all dyes. Five protein gel stains were used: Coomassie® Fluor Orange, SYPRO® Ruby, SYPRO® Tangerine, SYPRO® Red, and SYPRO® Orange.

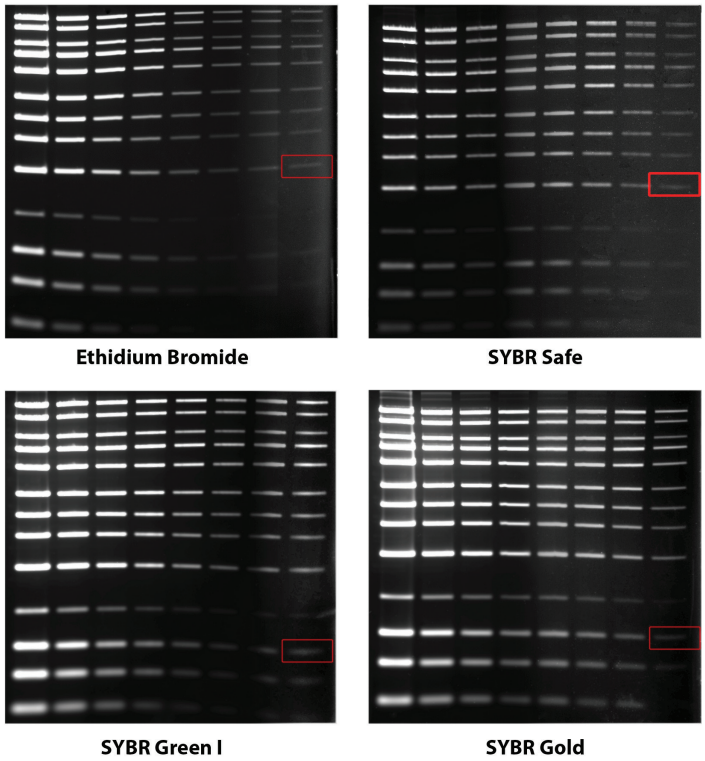

Figure 1. Agarose gels stained with ethidium bromide, SYBR Safe, SYBR Green I and SYBR Gold, respectively. Red boxes indicated the bands used to determine limit of detection.

Gel Imaging. Imaging was done with the FluorChem Q using the most appropriate combination of excitation and emission settings, including UV trans-illuminator for UV-excitation and an LED light source for visible wavelengths (Table 1).

Channel	Excitation	Emission Filter	Compatible Dyes
Blue	475/42 nm	537/35 nm	SYBR Green, SYBR Gold,SYBR Safe, SYPRO Ruby, SYPRO Orange, Cy2
Green	534/30 nm	606/68 nm	SYPRO Red, Cy3
Red	632/22 nm	699/62 nm	Cy5
UV	302nm	537/35nm, Orange	Coomassie FluorOrange, SYPRO Tangerine, SYBR Gold, SYBR Safe
UV	365nm	537/35nm, Orange	EtBr, SYBR Green

Table 1. Spectral characteristics of FluorChem Q filter sets and compatible dyes.

To reduce the amount of background fluorescence and fluorescent residues on the gel, it is highly recommended that users take extra care to handle gels properly for fluorescent imaging. Most gloves commonly used for lab work leave fluorescent residue on gels. Washing gloves prior to handling gels or using polyethylene "no fluorescence gloves" in

place of regular gloves will reduce fluorescent residue. Low fluorescence imaging sheets are also a useful accessory for fluorescent gel imaging.

Cell Biosciences products used:

- FluorChem Q

Other materials required:

- 1% Agarose (Amresco J234)
- TAE buffer
- Mini-submerged system (Bio-rad 170-4467)
- 1kB DNA ladder (Fisher BioReagents BP2278100)
- Ethidium bromide (Invitrogen E3565)
- SYBR Safe (Invitrogen S33102)
- SYBR Green I (Invitrogen S7567)
- SYBR Gold (Invitrogen S11494)
- 8-16% linear gradient Ready Gel (Bio-Rad 161-1223)
- Protein molecular weight standards (Invitrogen P6649)
- Standard Laemmli loading buffer
- SYPRO Red (Invitrogen S6653)
- SYPRO Orange (Invitrogen S6650)
- Coomassie FluorOrange (Invitrogen C33250)
- SYPRO Ruby (Invitrogen S12001)
- SYPRO Tangerine (Invitrogen S12010)
- No fluorescence gloves
- Low fluorescence imaging sheets

Results and Discussion

The FluorChem Q's DNA gel imaging performance was tested by imaging four different DNA gels each stained with one of four commonly used dyes: ethidium bromide, SYBR Green I, SYBR Gold and SYBR Safe. With the FluorChem Q imaging system, using the UV trans-illuminator set at 365nm and the Orange filter, visualization of 0.11ng of the 1,500bp DNA fragment stained with ethidium bromide was achieved. The SYBR Safe stained gel was imaged with the blue channel which allowed visualization of 0.11ng of the 1,500bp band. Using SYBR Green I dye and the UV trans-illuminator set at 365nm with the 537nm filter allowed visualization of 0.05ng of the 700bp band. Similarly, for the gel stained with SYBR Gold excited at 302nm and imaged with 537nm filter, detection of 0.05ng of the 700bp DNA fragment was accomplished (Figure 1, Table 2).

The FluorChem Q imager is also capable of detecting minute levels of protein. To demonstrate this point, SDS-PAGE gels stained with five commonly used protein gel stains were used: SYPRO Ruby, SYPRO Orange, SYPRO Red, Coomassie Fluor Orange, and SYPRO Tangerine. The FluorChem Q detected 2ng of protein on the gels stained with Coomassie Fluor Orange, and SYPRO Tangerine. The protein gels stained with SYPRO Ruby and SYPRO Orange allowed for detection of 1ng or less of the sample. Finally, 4ng of sample was detected on the protein gel stained with SYPRO Red (Figure 2, Table 2).

The FluorChem Q offers users the flexibility to try multiple combinations of excitation sources and emission filters in order to record the best possible gel image. A few of the different combinations possible are listed in Table 2. For example, gels stained with SYBR Gold can be visualized equally well using either the UV trans-illuminator set at 302nm with the 537nm filter or with the blue channel. This flexibility has many advantages. First, using visible light for excitation (475nm) of a stained DNA sample is better for the sample than exposure to UV

Stain	Excitation Source	Emission Filter	Integration Time (s)	Amount of sample detected (ng)
Ethidium Bromide	UV-365	EtBr	16	0.11
SYBR Green	UV-365	537/35nm	16	0.05
	475/42nm	537/35nm	16	0.05
SYBR Gold	UV-302	537/35nm	8	0.05
	475/42nm	537/35nm	16	0.05
SYBR Safe	475/42nm	537/35nm	16	0.11
	UV-302	537/35nm	16	0.23
SYPRO Ruby	475/42nm	606/68nm	8	0.5
SYPRO Orange	475/42nm	537/35nm	16	1
SYPRO Red	534/30nm	699/62nm	33	4
Coomassie FluorOrange	UV-302	Orange	4	2
SYPRO Tangerine	UV-302	Orange	8	2

Table 2. Conditions used to capture gel images on the FluorChem Q CCD imager. The excitation source and emission filters combinations used in this study are listed in this table. Users may find that other combinations also work well.

light. Over time, exposure of DNA to UV can cause damage such as knicking and crosslinking. 475nm excitation does not damage DNA, thus protecting the sample for future experiments. In addition to being safe for samples, 475nm excitation often has lower background levels than UV excitation. Photobleaching is also much lower when visible light is used for excitation. And finally, many of the annoying particles and lint often present in agarose are very bright when excited by UV, but are almost invisible when excited with 475nm. Thus, the flexibility of the FluorChem Q gives scientists the tools they need to obtain clear, precise and highly sensitive gel images.

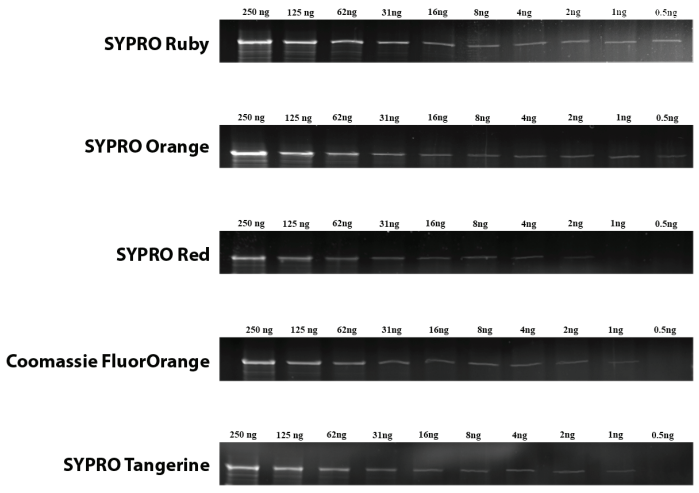

Figure 2. SDS-PAGE gels stained with SYPRO Ruby, SYPRO Orange, SYPRO Red, Coomassie FluorOrange, and SYPRO Tangerine, respectively.

Conclusion

The FluorChem Q provides a powerful means to image both multicolor Westerns as well as standard DNA and protein gels. The results show the FluorChem Q imaging system capable of achieving high levels of sensitivity in both DNA and protein imaging. The sensitivity of the system is reflected in a detection limit of less than 1ng of sample in both DNA and protein gels, which is comparable to the detection limits given by the manufacturers of the various dyes and stains used in this study (1-7).

Acknowledgement

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