

Fluorescent Protein Gel Stains

Typhoon Variable Mode Imager

Key words: fluorescence, imaging, Typhoon, protein gels, SYPRO gel stains

Fluorescent protein gel staining, when combined with an appropriate imager, is a sensitive and quantitative approach for protein analysis. Fluorescent gel stains also offer advantages such as ease of use, sample stability, and safety (1).

SYPRO™ Orange, Red, Ruby, and Tangerine protein gel stains are one-step fluorescent stains optimal for rapid and efficient staining of one-dimensional (1-D) protein gels. These stains provide sensitivity that is equivalent to the silver staining method for 1-D gels (2) and enable protein detection that is not affected by the presence of nucleic acids and lipopolysaccharides. SYPRO Ruby stain is also suitable for two-dimensional (2-D) protein gel staining (2, 3).

The Typhoon™ Variable Mode Imager provides sensitive fluorescence detection for protein gels stained with the SYPRO stains. The Typhoon 8600 and 9200 series have two excitation sources for fluorescence imaging: a green (532 nm) and a red (633 nm) laser. The Typhoon 9400 series has an additional blue laser with two excitation lines (457 nm and 488 nm).



Products used

Typhoon 8600	63-0027-96
Typhoon 9200	63-0038-49
Typhoon 9210	63-0038-51
Typhoon 9400	63-0038-53
Typhoon 9410	63-0038-55
EPS 301 Power Supply	18-1130-01
Hofer™ miniVE Electrophoresis System	80-6418-77
SYPRO Orange Protein Gel Stain	RPN5801
SYPRO Red Protein Gel Stain	RPN5803
SYPRO Tangerine Protein Gel Stain	RPN5805
Low fluorescence Glass Plate, 3 mm thick	63-0028-92

PlusOne™ reagents

Acrylamide IEF	17-1300-01
Bromophenol Blue (BPB)	17-1329-01
Dithiothreitol (DTT)	17-1318-01
Glycerol (87% w/w)	17-1325-01
Glycine	17-1323-01
N,N'-Methylene-bisacrylamide	17-1304-01
Sodium Dodecylsulphate (SDS)	17-1313-01
Tris	17-1321-01

Other materials required

- SYPRO Ruby Protein Gel Stain (Molecular Probes)
- Bovine serum albumin (BSA) standard
- Methanol
- Glacial acetic acid
- PBS buffer
 - 10 mM phosphate, pH 7.4
 - 2.7 mM potassium chloride
 - 137 mM sodium chloride
- Treatment buffer (TB)
 - 62.5 mM Tris-Cl, pH 6.8
 - 2% SDS
 - 10% Glycerol
 - 0.01% BPB

Protocol

1

Preparing the sample

- 1.1. Prepare two-fold serial dilutions of protein sample in TB/DTT (0.1 M) buffer.

2

Performing the gel electrophoresis

- 2.1. Prepare 1-mm-thick gels with a 4% stacking gel and a 10% resolving gel using the Hoefer miniVE Electrophoresis System.
- 2.2. Load 5 μ l of the prepared sample onto the gel.
- 2.3. Perform electrophoresis using the EPS 301 Power Supply for 2 h at 12.5 mA constant current per gel.

3

Staining and destaining

- 3.1. The staining and destaining of the gels should be performed with gentle shaking.

For SYPRO Orange or Red, stain the gel for 30 min in 50 ml of the stain diluted to 1:5000 in a 7.5% (v/v) acetic acid solution. Destain the gel for 15 min in 50 ml of a 7.5% (v/v) acetic acid solution.

For SYPRO Ruby, stain the gel in 50 ml of the working stain solution for at least 12 h. Rinse the gel briefly with deionized water. Destain for 30 min in 50 ml of a solution of 10% (v/v) methanol and 7% (v/v) glacial acetic acid.

For SYPRO Tangerine, stain the gel for 40 min in 50 ml of the stain diluted to 1:5000 in PBS buffer. Destain the gel for 30 min in 50 ml of PBS buffer.

4

Imaging

- 4.1. The gel can be imaged on a 3-mm-thick low-fluorescence glass plate using the +3 mm focal plane setting. The glass plate helps to keep the gel intact permitting future analysis steps. The plate also helps to protect the Typhoon glass platen from temporary contamination by the SYPRO stains, which is a problem common with this type of stain on all imagers. A thorough cleaning of the glass plate is recommended immediately after scanning (see 4.3).

A thin layer of deionized water between the glass plate and the platen will minimize the appearance of interference patterns. Pour sufficient deionized water onto the platen to form a pool and then gently place the low-fluorescence glass plate on top of the water. Avoid trapping any bubbles between the glass plate and the platen. Pour a small amount of deionized water onto the glass plate. Place the gel on top of the water. Avoid bubble formation between the gel and the glass plate.

Alternatively, the gel can be imaged by using “platen” for the focal plane setting and directly placing it on the platen on a small amount of deionized water. Avoid bubble formation between the gel and the platen. A thorough cleaning of the platen is recommended immediately after scanning (see 4.3).

- 4.2. Choose the Typhoon settings as recommended in Table 1. In addition, choose an appropriate PMT voltage setting (450–800 V) and focal plane setting (see 4.1), as well as the following parameters:

Pixel size – 200 μ m

Sensitivity – Normal

For more details about setting up the scanner control and other practical aspects of using Typhoon, please refer to *Typhoon Instrument Guide*.

- 4.3. After scanning, use a soft, lint-free cloth dampened with deionized water to clean the glass plate or the platen and, if necessary, the sample lid. Repeat the procedure with 75% ethanol. Rinse again with deionized water to remove any possible fluorescent residue from the ethanol. If the glass plate or the platen is still dirty, clean with a 5–10% hydrogen peroxide solution followed by extensive rinsing with deionized water to remove residual hydrogen peroxide.

Table 1. Recommended Typhoon settings for imaging protein gels treated with SYPRO stains.

Gel stain (ex, em)	Laser	Emission filter
SYPRO Orange (470 nm, 570 nm)	Green (532 nm)/Blue (488 nm)	555BP20
SYPRO Red (550 nm, 630 nm)	Green (532 nm)	610BP30
SYPRO Ruby (450 nm, 610 nm)	Green (532 nm)/Blue (457 or 488 nm)	610BP30
SYPRO Tangerine (490 nm, 640 nm)	Green (532 nm)/Blue (488 nm)	610BP30

Results

An example of BSA detection using SYPRO Ruby imaged by the Typhoon 457 nm, 488 nm, and 532 nm laser lines is shown in Figure 1. Using the experimental conditions described in this application note, similar imaging results were achieved using any of these three laser lines for SYPRO Ruby detection of BSA.

It is important to note that the detection limits of these post-staining gel applications are not limited by the performance of the Typhoon imager. This is strongly supported by the fact that using an excitation wavelength closer to a stain's excitation maximum or increasing laser power does not significantly improve the detection limits of these applications. For example, judging from the excitation maximum of SYPRO Ruby at 450 nm, using the 457 nm laser line to excite SYPRO Ruby is, in theory, more appropriate than using 532 nm. However, the results in this application note demonstrate that 457 nm and 532 nm offer essentially similar imaging results for SYPRO Ruby stained BSA gels (Fig 1). The major factors limiting sensitivity are the background staining of the gel, the imperfect stain/protein binding stoichiometry, and other experimental factors.

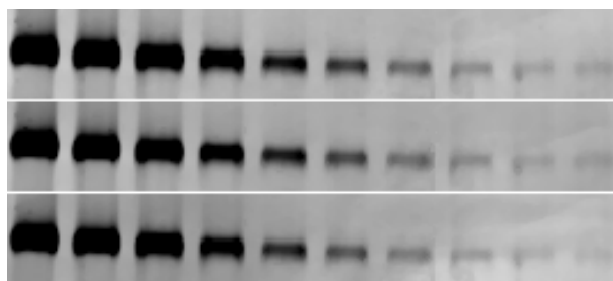


Fig 1. SYPRO Ruby detection of BSA imaged by Typhoon 457 nm (top image), 488 nm (middle image), and 532 nm (bottom image) laser lines with the 610BP30 emission filter. The amount of BSA per lane ranges from 512 ng to 1 ng, in 2-fold serial dilutions. From left to right, lanes 1 to 10 contain: 512 ng, 256 ng, 128 ng, 64 ng, 32 ng, 16 ng, 8 ng, 4 ng, 2 ng, and 1 ng of BSA.

Table 2 summarizes the limit of detection (LOD) and linear ranges for BSA stained with SYPRO Orange, Red, Ruby, and Tangerine using the Typhoon instrument settings listed in Table 1. Note that Typhoon offers a wide linear dynamic range of five orders of magnitude (from count 1 to 100 000). Nevertheless, the linear range of protein detection by the SYPRO stains (Fig 2) is typically limited to three orders of magnitude by the factors mentioned in the previous paragraph. This also suggests that Typhoon has the potential to offer even better imaging results when improved fluorescence staining methods become available.

Table 2. Summary of Typhoon LOD and linear range for BSA stained with SYPRO Orange, Red, Ruby, and Tangerine

Gel stain	Typhoon LOD (ng/band)	Linear range
SYPRO Orange	2	~1000 fold
SYPRO Red	2	~1000 fold
SYPRO Ruby	1	~2000 fold
SYPRO Tangerine	2	~1000 fold

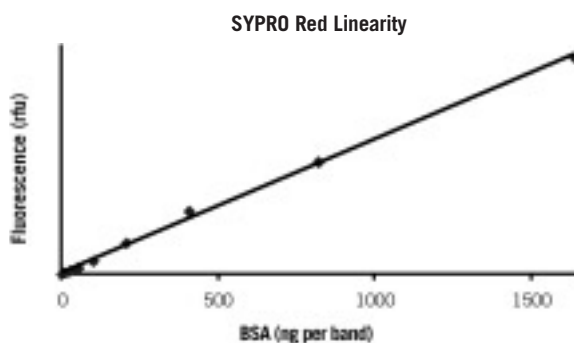


Fig 2. Fluorescence intensity of BSA bands stained with SYPRO Red versus BSA concentration (ng per band). Fluorescence refers to the integrated relative fluorescence units (rfu) from ImageQuant™ volume analysis using rectangle objects and local median background correction. The linear fit to the data has an R^2 value of at least 0.99.

Conclusions

Typhoon offers high sensitivity and a wide linear range for the quantitation of fluorescently stained protein gels. The wide range of excitation sources and emission filters combined with the highly efficient detection optics, are suitable for the detection of SYPRO Orange, Red, Ruby, and Tangerine protein stains. The LOD for SYPRO Orange, Red, and Tangerine is 2 ng of BSA per band. SYPRO Ruby has an LOD of 1 ng of BSA per band.

References

1. *Fluorescence Imaging: principles and methods*, Amersham Biosciences, code number 63-0035-28, (2000).
2. Patton, W. F., *Electrophoresis*, **21**, 1 123–1 144 (2000).
3. Application Note 69, *Imaging Two-Dimensional Protein Gels Stained with SYPRO Ruby*, Amersham Biosciences, code number 63-0043-06, (2001).

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