

SCIENCE IMAGING SYSTEMS

Application Note

No. 4

Protein Detection with SYPRO® Orange (1)

FLA-2000

Introduction

Coomassie Brilliant Blue (CBB) has long been the primary stain for the detection of proteins in electrophoretic gels. Although the silver staining method, introduced in 1980, was rapidly accepted as another technique capable of providing high-sensitivity detection, the procedure is tedious and the results are not totally quantitative. The choice of whether to use CBB or silver staining is dictated by the nature of the sample being analyzed. For these reasons, scientists have long desired an alternative method for the detection of proteins in gels that is both highly sensitive and easy to use.

The SYPRO® Orange staining method, first reported in Analytical Biochemistry in 1996, has been described as a simple method very similar to CBB staining. The FLA-2000 is equipped with an SHG laser device whose wavelength of operation is 473nm and is very close to the optimum excitation wavelength of SYPRO® Orange, which lies at 472nm. As a consequence, the FLA-2000 is ideally configured to fully exploit the spectral characteristics of this new dye.

This Application Note deals with the fundamentals of working with SYPRO® Orange.

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1. SYPRO® Orange Stain of Gels
2. Comparison of Different Staining Methods
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Summary

- Sample preparation is easier with SYPRO® Orange than with conventional silver and CBB staining methods.
- Sensitivity is comparable to, or better than, silver staining.
- The limit of detection using SYPRO® Orange was determined to be 500pg.

1 SYPRO® Orange Stain of Gels

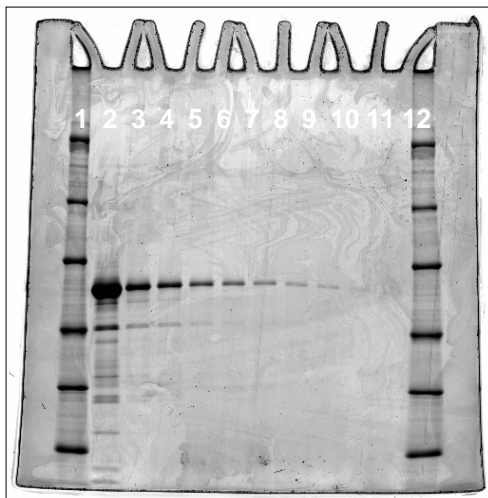


Fig. 1-1

Fig.1-1 SYPRO® Orange Stained Electrophoresis Gel of Bovine Serum Albumin(BSA)

Electrophoresis was carried out on lanes 2-11 in the following amounts of 1µg, 100ng, 50ng, 20ng, 10ng, 5ng, 2ng, 1ng, 500pg and 200pg. A molecular weight marker was applied to lanes 1 and 12.

The sample was subjected to electrophoresis at a constant current of 20mA.

The digital image was generated by scanning the gel with the FLA-2000 under the following conditions:

- Gradation : 65536(16bit)
- Resolution : 50µm
- Sensitivity : F10
- Latitude : 5
- Sample Mode : Fluor. 473nm
- : Y520 Filter

Excitation/Emission Spectra of SYPRO® Orange

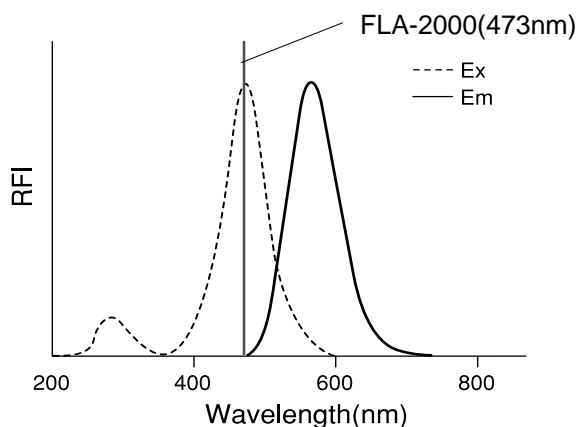


Fig. 1-2

RFI

Relative fluorescence intensity

Fig.1-2 Excitation/Emission Spectra of SYPRO® Orange Stained Protein

The maximum excitation wavelength is 472nm and the maximum emission wavelength is 570nm.

(Ex : Excitation spectrum, Em : Emission spectrum)

Note that FLA-2000 is using excitation wavelength 473nm, new SHG laser device, which is just fit for SYPRO® Orange with excitation maximum at 472nm.

Amount of Protein vs. Fluorescence Intensity

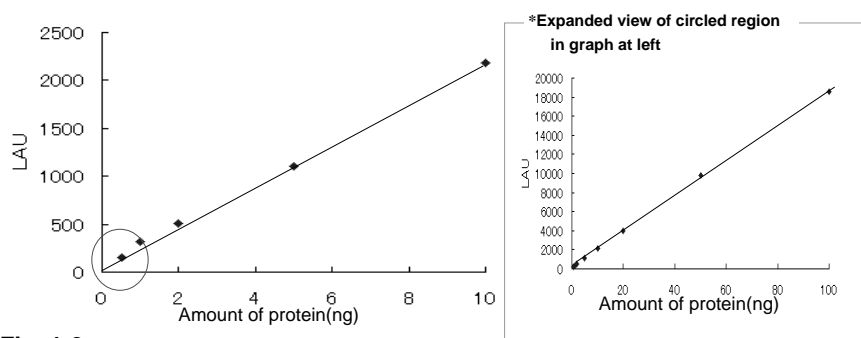


Fig. 1-3

Fig.1-3 Quantitative Fluorescence Response of SYPRO® Orange

Left : Amount of Protein vs. Fluorescence Intensity

Right : Expanded view of circled region of graph at left.

The data were obtained using the profile analysis feature of the MacBAS software for the FLA-2000. Background was subtracted.

(LAU: Linear Arbitrary Unit is the fluorescence intensity unit used in FLA-2000.)

2 Comparison of Different Staining Methods

The procedures are represented schematically in Fig.1-4 (below)

The limit of protein detection with SYPRO® Orange staining and the FLA-2000 was determined to be 500pg, comparable to the limits of detection when using silver staining. SYPRO® Orange staining is simpler and faster than either silver staining or CBB staining.

Fig.1-4 SYPRO® Orange Stained Electrophoresis Gel of BSA

Electrophoresis was carried out on lanes 2-11 in the following amounts of 1µg, 100ng, 50ng, 20ng, 10ng, 5ng, 2ng, 1ng, 500pg and 200pg. A molecular weight marker (Daiichi II, product of Daiichi Pharmaceutical Co., Ltd.) was applied to lanes 1 and 12.

SYPRO® Orange staining was conducted at a dilution of 1:5000.

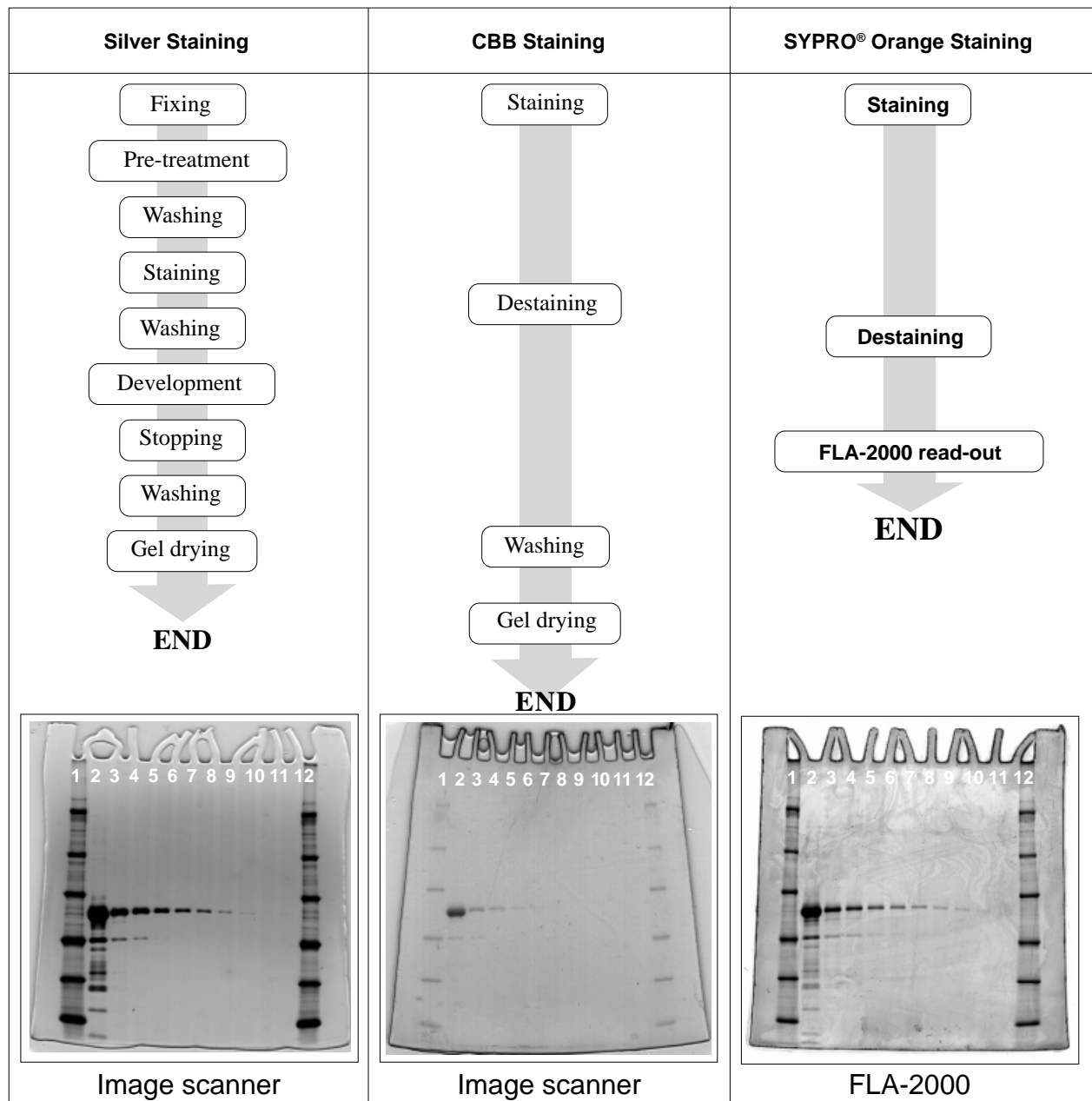
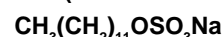


Fig. 1-4

3 Reagent Properties

Name	SYPRO® Orange
Classification	Fluorescent dye
Mechanism of Action	Thought to produce fluorescence by association with protein-SDS complex.
Sample	Protein, glycoprotein, lipoprotein
Storage	Keep refrigerated. Dispose of staining solution after maximum of four uses.
Caution	Wear gloves when handling.
Manufacturer	Molecular Probes Inc.

SDS(Sodium Dodecyl Sulfate)



SDS is a surface active agent added to the polyacrylamide gel when conducting electrophoresis. By denaturing proteins, it ensures migration proportional to molecular weight.

4 References

On silver staining

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3. Poehling, H. M., Neuhoff, V. ,Visualization of Proteins with a Silver Stain : A Critical Analysis, *Electrophoresis* ; 2, 141-147, (1981)

On SYPRO® Orange

4. Steinberg, T.H., Haugland, R.P., Singer, V.L., Applications of SYPRO Orange and SYPRO Red Protein Gel Stains, *Analytical Biochemistry* ; 239, 238-245, (1996)
5. Steinberg, T.H., Jones, L.J., Haugland, R.P., SYPRO Orange and SYPRO Red Protein Gel Stains: One-Step Fluorescent Staining of Denaturing Gels for Detection of Nanogram Levels of Protein, *Analytical Biochemistry* ; 239, 223-237, (1996)

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