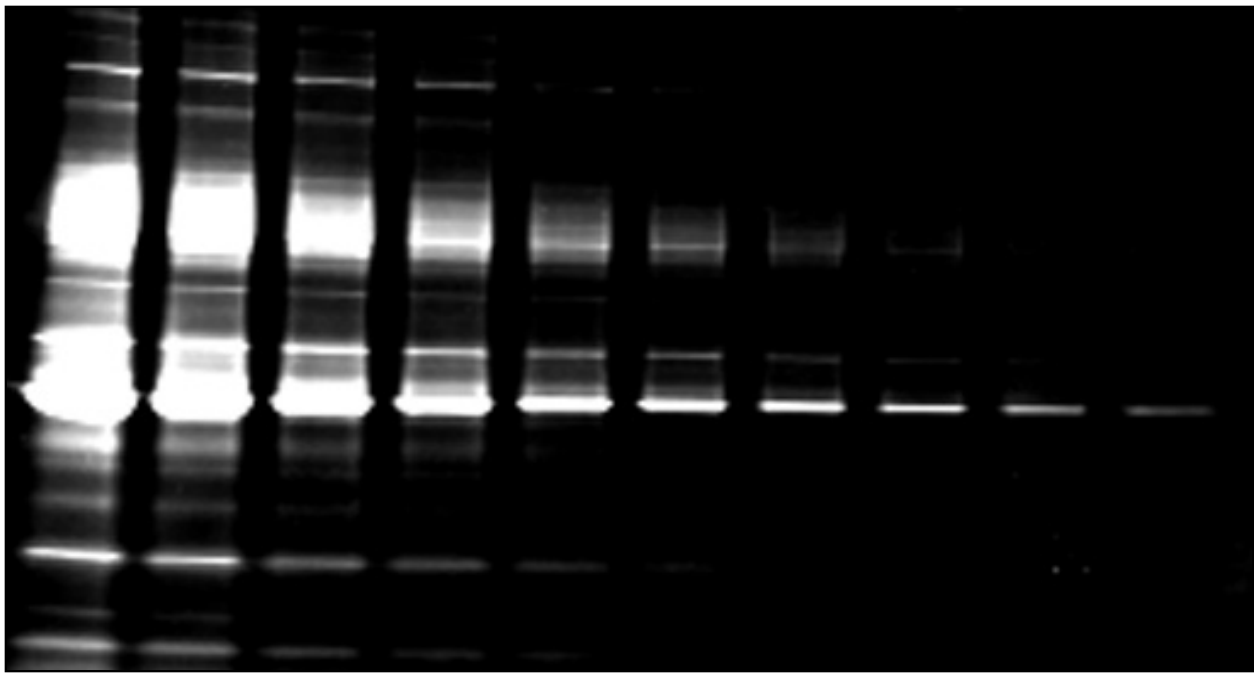
HITACHI
Genetic Systems
Hitachi Software Engineering Co., Ltd.

APPLICATION NOTE

FMBIO® Detection of SYPRO® Red Dye Stained Proteins in Polyacrylamide Gels



1 **Two-fold dilution series of human serum stained with SYPRO® Red dye.** Serum was serially diluted (Lane 1 = 13.0 µg) and loaded onto a 4-20%, 1-mm Tris-Glycine gel (NOVEX) and electrophoresed for 90 minutes in a 1X Tris Glycine running buffer[1] with 0.05% SDS. Gels were stained in SYPRO® Red dye (1:5000 in 7.5% acetic acid) with gentle agitation. To reduce background, a destaining step was performed in which gels were gently agitated in 7.5% acetic acid for 45 minutes. The image was acquired on the FMBIO® II Fluorescence Imaging System using a 625/15 nm filter at 100% photomultiplier tube sensitivity, with 256 repeats and a spatial resolution of 300 x 300 dots per inch.

Applications

SYPRO® Red protein gel stain was developed to provide fast, simple, sensitive staining of proteins in electrophoretic gels. The FMBIO® II Fluorescence Imaging System can be used to image the following types of protein gels stained with SYPRO Red dye:

- SDS-denaturing Gels
- Native Gels
- 2-D Gels

Benefits

Scanning SYPRO Red dye stained gels on the FMBIO II Fluorescence Imaging System provides a number of benefits compared to Coomassie® Brilliant Blue and silver staining[2,3].

- Data acquisition with the FMBIO is fast and easy.
- Gel fixation is unnecessary, resulting in efficient transfer of protein bands to membranes for western blot analysis.
- Staining does not modify antigenic sites, so they are left available for antibody binding.
- Polypeptide sequence and degree of glycosylation do not affect staining intensity and quality.
- Detection sensitivities are comparable to silver staining and are higher than Coomassie staining.
- Nucleic acids found in protein mixtures from cell or tissue extracts are not stained.
- SYPRO Red stain is optimally matched to the FMBIO laser for maximum imaging sensitivity, with minimum background fluorescence.
- The combination of the FMBIO and SYPRO Red stain results in greater dynamic range than instant film or CCD cameras.
- FMBIO Analysis Software allows a wide variety of quantitation capabilities.
- Digital images can be created, published, and archived, eliminating the need to dry and save gels.

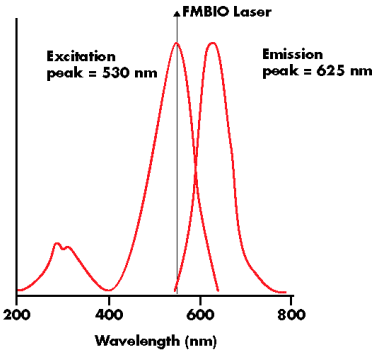
Sensitivity

625 pg/band

Dynamic Range

2.2 orders of magnitude (625 pg/band to 100 ng/band)

Dye Spectra



Tips for FMBIO Imaging

1. Many factors contribute to the success of protein detection. Variables include: gel type and concentration, sample preparation, band size, dye concentration, staining time, destaining time, gray level settings, and laser focusing point. Further optimization of protocols may result in higher detection sensitivity.

2. For best scanning results, avoid dust specks by using only powderless gloves, rinsing gloves with distilled water, thoroughly cleaning all containers used for staining, and filtering all buffers and solutions with a 0.45µm filter.

3. For lowest background, place the gel on a low-fluorescence glass plate or sample holder when scanning. Do not scan on plastic wrap unless the wrap has been tested for low background fluorescence.

4. Keep solutions containing SYPRO Red stain covered by aluminum foil to prevent photobleaching of the dye by ambient light. Staining reagent diluted in buffer or acetic acid and stored protected from light at 4˚C may be reused for at least three months.

5. Before opening each vial, warm it to room temperature and then briefly centrifuge. If dye particles are present, they should be redissolved by briefly vortexing the tube after warming.

6. For detailed protocols on the usage, handling and disposal of SYPRO Red stain, see Molecular Probes Product Information Sheet TD005[4].

FMBIO Analysis Software provides easy-to-use features for image analysis:

- Band Sizing
- 1D Volume Calculation
- 1D Peak Height
- Lane Traces
- Spot Quantitation
- Background Subtraction
- Standard Curve
- Text and Other Annotation
- Gray Level Adjustment
- User-defined Scan Options

References

1. Ausubel, F.M. *et al.* (1992). Short Protocols in Molecular Biology, 2nd Ed., John Wiley and Sons, New York.

2. SYPRO® Protein Gel Stains. (1996). Molecular Probes Product Information Sheet MP 6650.

3. Ishino Y., *et al.* (1992). Practical Applications in Molecular Biology of Sensitive Fluorescence Detection by a Laser-excited Fluorescence Imager Analyzer. *BioTechniques* **13**, 936.

4. SYPRO® Protein Gel Stains (1997). Molecular Probes Product Information Sheet TD 005.

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