



Abstract

Optimum performance of SYPRO® Ruby protein gel stain required an overnight incubation in stain solution. We developed a 90 minute microwave protocol that increased sensitivity and lowered background, while preserving the other performance characteristics of SYPRO® Ruby stain in 1-D and 2-D SDS-PAGE gels. Following a 30 minute fixation step, gels were placed in stain solution, briefly heated in a microwave, incubated for 30 minutes, and then destained for 30 minutes. Using this protocol, protein bands containing 0.25 ng protein could be detected. Protein samples were taken from gels following the microwave or overnight protocols and analyzed by MALDI mass spectrometry. Protein yield and quality were not changed by the microwave protocol. The entire microwave protocol requires 90 minutes to complete and is compatible with both Pro-Q® Emerald glycoprotein dye and Pro-Q® Diamond phosphoprotein dye staining for multiplexed proteomic platform analysis.

Summary of SYPRO® Ruby Gel Stain Protocols

	Reagent	Overnight Protocol	Microwave Protocol
Fix	50% methanol, 7% acetic acid	100 ml, 30 min	100 ml, 15 min
Stain	SYPRO® Ruby gel stain	75 ml, overnight (room temperature)	75 ml, microwave 30 seconds, agitate 5 minutes, microwave 30 seconds, agitate 23 minutes (30 minutes total) (80-85 °C)
Wash	10% methanol, 7% acetic acid	100 ml, 30 min	100 ml, 30 min
Total Time		~18 hours	90 minutes
Hands on time		10 minutes	15 minutes

Table 1.

Comparison of SYPRO® Ruby Protocols on 1-D Gels

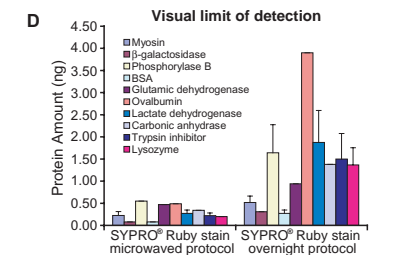
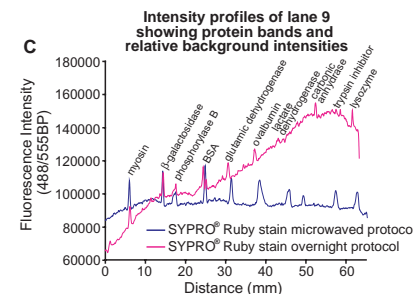
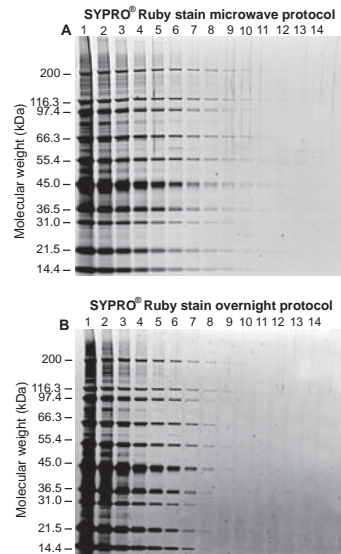


Figure 1. A two-fold dilution series of Mark 12™ unstained standards (Invitrogen) supplemented with ovalbumin were separated on 4-12% Bis-Tris NuPAGE gels. Duplicate gels were stained with SYPRO® Ruby gel stain according to the overnight or microwave protocol described in Table 1. Image acquisition was done with a Bio-Rad FX imager using 488 nm excitation and a 555 nm long-pass emission filter. Lane 9 contains approximately 1 ng of each protein. Error bars in Figure 1D represent the standard deviation for quadruplicate gels.

- With room temperature SYPRO® Ruby dye staining, low sensitivity could be achieved with a 3-4 hour incubation, but optimal results were seen with overnight incubation.
- Microwaving SYPRO® Ruby stain to 80-85 °C with a 30 minute incubation resulted in increased sensitivity (0.25-0.5 ng) compared to the overnight stain protocol (1-4 ng).
- Gels stained in SYPRO® Ruby dye following the microwave protocol showed lower background staining and reduced speckling artifacts compared to the overnight protocol.

Multiplexing with Pro-Q® Diamond Phosphoprotein Stain and SYPRO® Ruby Stain in 2-D Gels

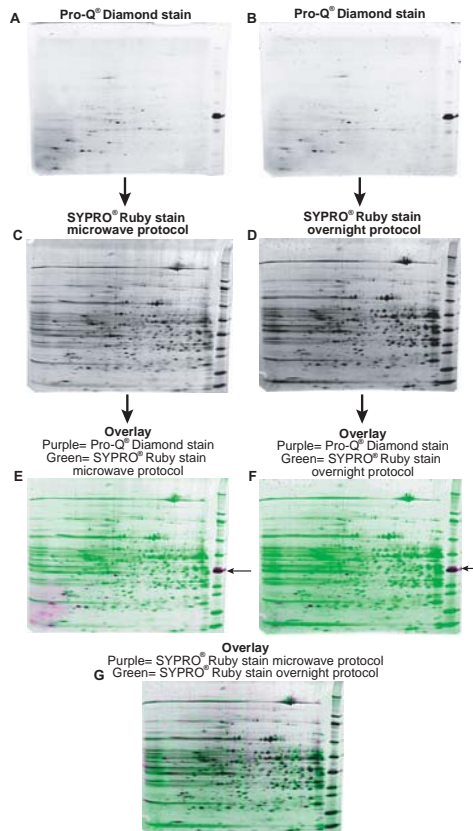


Figure 2. A sample of rat liver lysate (20 µg) was separated by pI on pH 4-7 IPG strips and then by molecular weight on 4-12% Bis-Tris NuPAGE gels using the Zoom Benchtop Proteomics System (Invitrogen). Duplicate gels were stained with Pro-Q® Diamond phosphoprotein gel stain and then with SYPRO® Ruby gel stain according to the overnight or microwave protocol. Images were pseudo-colored and overlaid with Z3 software (Compugen, Tel Aviv, Israel). Proteins detected with both dyes appear black in the overlaid images. Ovalbumin, the only phosphoprotein present in the marker mix, is the only marker protein stained by Pro-Q® Diamond stain (arrow).

- Similar total protein staining patterns were obtained with 2-D gels post-stained with SYPRO® Ruby stain using the microwave protocol or the overnight protocol following Pro-Q® Diamond phosphoprotein dye staining.
- The pattern of phosphoprotein spots on 2D gels was nearly identical between protocols.

Comparison of the Detection Sensitivity and Linear Dynamic Range of Various Proteins

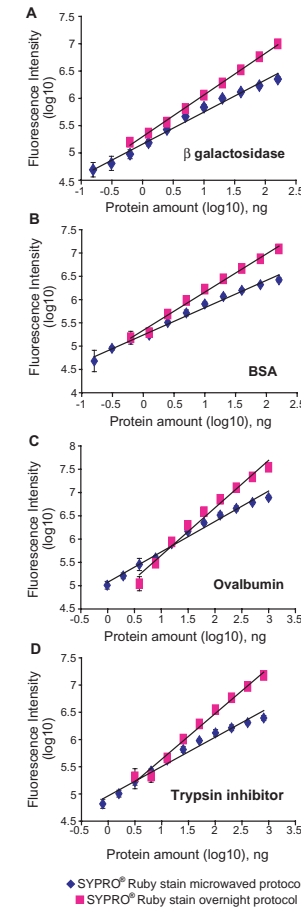


Figure 3. Plots of the log10 fluorescence intensity vs. log10 protein amount show the fluorescence response across the full concentration range. All data points were determined in quadruplicate.

- Both SYPRO® Ruby dye staining protocols showed 2.5 to 3 log linear dynamic range.
- Overnight staining with SYPRO® Ruby stain gave brighter staining of abundant proteins but showed lower sensitivity compared to the microwave protocol.

MALDI Mass Spectrometry Comparison

	BSA	Myoglobin
pI	5.60	7.36
MW	66432.96	16951.48
SWISS PROT accession no.	P02769	P68082
Stain	Sequence Coverage	
SimplyBlue	38%	88%
SYPRO® Ruby Overnight Protocol	40%	81%
SYPRO® Ruby Microwave Protocol	35%	81%

Table 2.

MALDI Mass Spectrometry Comparison

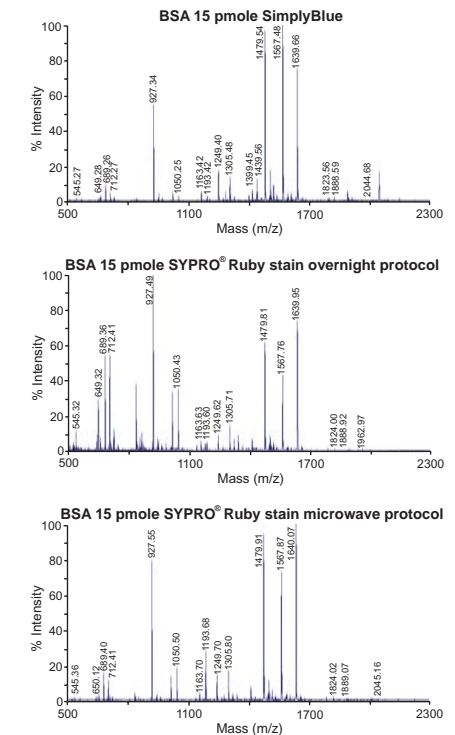


Figure 4. Peptide fragments of BSA and myoglobin were analyzed from proteins extracted from gels that had been stained with SYPRO® Ruby dye using the microwave or the overnight protocols and from gels stained with Coomassie blue (Simply Blue™ SafeStain, Invitrogen). Two or 15 pmol samples were then analyzed on an ABI Voyager DE STR mass spectrometer. The % sequence coverage of the two proteins was determined (Table 2).

- The percent sequence coverage of BSA and myoglobin after SYPRO® Ruby dye staining was comparable to Coomassie blue stain whether or not the microwave treatment was used.
- There was no evidence for protein fragmentation due to the microwave treatment.

Conclusions

- The SYPRO® Ruby microwave stain protocol reduces the time required for optimum staining performance to 90 minutes.
- The microwave stain protocol improves sensitivity, lowers background staining, and speckling artifacts compared to the overnight stain protocol.
- Other performance advantages with SYPRO® Ruby staining over other total protein stains, such as a low nanogram to microgram quantitation range, multiplexing capability, and mass spectrometry compatibility are not affected by microwaving the stain solution.

Acknowledgments

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References

Nesatyy, V.J., Dacanay, A., Kelly, J.F., Ross, N.W. (2002) Rapid Commun. Mass Spectrom. 16: 272-280.