

# LSUHSC Proteomics Core Facility

## Applications Newsletter

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### The Choice of stain for 2D-gel electrophoresis

The Proteomics Core Facility offers several stains for visualization of 2-D gels: Bio-Safe™ (a colloidal coomassie G-250), Silver Plus™, and a fluorescent stain, Sypro Ruby™. In this issue, we compare the three stains and their compatibility with mass spectrometry analysis for protein identification (see last issue).

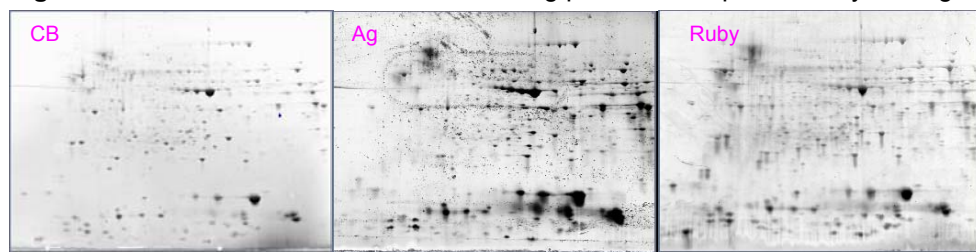
#### Method

Protein extracts of dog heart tissue were separated by the Bio-Rad Protean IEF/Criterion PAGE 2D gel electrophoresis system. Three identical gels were stained with three stains according to the manufacturers' instructions. All gel images are scanned by GS-800 (except Ruby stained gels by GE Typhoon) and analyzed by Bio-Rad PDQuest. Selected *matched* spots were subjected to protein ID, accomplished by our state-of-the-art Applied Biosystems MALDI-TOF-TOF and in-house Mascot database search engine.

#### Results

##### Gel Spot Quantitation

**Figure 1.** Illustration of the variation in staining pattern and spot intensity among four different gel stains.



Gel images shown Figure 1 from left to right are stained with BioSafe™ (CB), Silver Plus™ (Ag), and Sypro Ruby™ (Ruby). It is clear that the most well-defined spots are visualized in Sypro Ruby stained gel. Silver

stain, traditionally considered the “sensitive” stain, has a smallest dynamic range of quantification. In Ag, the most abundant protein spots are overexposed and infuse into the surrounding spots, but low abundant protein spots are not displayed, which causes the lowest number of detected spots. In Table 1, the Correlation Coefficients indicate that BioSafe and Sypro Ruby yield similar quantitative results.

**Table 1** Comparative Spot Analysis and Match Rates

| Gel Stain          | Spots | Matched | Match Rate | Corr Coeff |
|--------------------|-------|---------|------------|------------|
| <b>BioSafe</b>     | 389   | 386     | 100%       | 1          |
| <b>Silver Plus</b> | 319   | 259     | 67%        | 0.712      |
| <b>Sypro Ruby</b>  | 579   | 348     | 90%        | 0.877      |

Note:  
Match Rate = (# of Matched of this set) / (# of Matched in the BioSafe set).  
Correlation Coefficient: the relatedness of quantities of all matched protein spots between this gel and BioSafe.

#### Mass Spectrometry Compatibility

Abundant proteins from all three gels give similar quality of the mass spectra and similar database search results (high protein score). However, if the protein quantity is lower, BioSafe will most likely give the best mass spectra and yield the highest protein score. Sypro Ruby is the second best as shown in Table 2.

| Protein ID              | BioSafe | Silver Plus | Sypro Ruby |
|-------------------------|---------|-------------|------------|
| creatine kinase         | 559     | 509         | 512        |
| cytochrome c oxidase VI | 270     | 85          | 166        |
| lactate dehydrogenase B | 287     | 67          | 258        |

**Table 2** Comparison of protein Scores of the selected identical gel spots stained with three different stains.

#### Conclusion

We recommend Sypro Ruby for image analysis. For sequential protein ID, BioSafe is the best choice.

\*This work was presented in the 53<sup>rd</sup> ASMS meeting, San Antonio, TX, 6/4/05-6/10/05. The whole poster is available in the Proteomics Web site.

\*\*Please note that the service fees will be adjusted slightly in the 2005-2006 fiscal year.