Yun Khoon Liew[1]
Vasanthakumari Neela[1]
Rukman Awang Hamat[1]
Syafinaz Amin Nordin[1]
Pei Pei Chong[2]

[1]Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, Serdang, Malaysia
[2]Department of Biomedical Sciences, Universiti Putra Malaysia, Serdang, Malaysia

Received July 17, 2012
Revised September 14, 2012
Accepted September 21, 2012

Short Communication

Modified silver staining in 2DE improves protein detection even at extremely low sample concentration

The typical concentration of protein loaded varies from 0.13 to 1.40 μg/μL for a classical silver staining method in 2DE gel. Here, we present a simple modified classical silver staining method by modifying the silver impregnation and development reaction steps. This modified method detects the protein spots at extremely low loaded concentrations, ranging from 0.0048 to 0.0480 μg/μL. We recommend this modified silver staining as an excellent method for the limited biological samples used for silver-stained 2DE analysis. Altogether, the protocol takes close to two days from first dimension separation to second dimension separation, followed by silver staining, scanning, and analysis.

Keywords:
2DE / Protein spots / Silver stain

DOI 10.1002/elps.201200380

2DE is a common tool for protein expression comparison and functional studies of large datasets of complex residual proteins mixture that often reflect the physiological status of a sample. Well-separated residual proteins on a 2DE gel can be detected by a number of existing staining techniques. The sensitivity of each staining technique varies. For example, fluorescence dyes (sypro, deep purple, and cyanine) exhibit a higher sensitivity than CBB staining method [1–3]. But, fluorescence dyes are more expensive compared to CBB and silver staining reagents. Generally, silver staining method detects protein at about 100 pg per spot, compared with CBB staining that only detects nanogram of protein in a spot [4]. Even though the Colloidal Coomassie Blue (CCB) protocol has been recognized as more sensitive than conventional CBB when applied in 2DE approach, the sensitivity of CCB is still lower than silver staining technique [5–7]. Considering the cost and the sensitivity factors, silver staining method still remains the best choice for direct visualization of protein in gel.

Like any other staining methods, silver staining method also requires a high total protein loading concentration onto 2DE gel with at least 0.13 μg/μL as shown in literature [8–13]. For example, 0.86 μg/μL of protein extracted from neutrophil was required to gain a clear 2DE gel image with large number of proteins even when more sensitive silver stain was used [13]. This could be a great challenge for limited source of biological protein-containing samples as adequate technical replications are needed in 2DE. Besides, complex mixture of protein contains enormous residual protein or polypeptide "abundance". Therefore, certain highly abundant protein spots may cluster or overlap with neighboring less abundant protein spots when high amounts of proteins are loaded onto 2DE gel. The prolonged development time in silver staining in order to detect less abundant protein spots, usually results in high background signals that affect the qualitative or quantitative analysis. Although some sample separation strategies, such as depletion of highly abundant proteins or protein-enrichment can overcome these problems, it could be expensive and time consuming and may also loose bound less abundant proteins. Taken together into consideration, identifying proteins of interest that are present at lower abundance still remains a great challenge in the field of proteomics.

Therefore, this paper is aimed to introduce a modified silver staining approach for the detection of protein spots at extremely low loaded protein concentration. Here, we chose *Staphylococcus aureus* exoproteins that comprise both small and moderately abundant protein spots to compare the classical and modified silver staining methods in 2DE gel with low loaded protein concentration. Briefly, the *S. aureus* was grown to postexponential phase and the secreted protein was precipitated by ice-cold ethanol-TCA method [14]. The precipitated pellet was then resuspended in rehydration buffer (8 M urea, 2 M thiourea, 2.0% w/v CHAPS, 0.2% Bio-Lyte 3/10 ampholytes v/v, 50 mM DTT). Concentration of the exoprotein was determined by RC-DC Protein Assay (Bio-Rad). A total of 0.048 μg/μL and 0.0048 μg/μL of solubilized protein-containing samples (*S. aureus* exoprotein) in 125 μl rehydration buffer were loaded onto 7 cm IPG strips (Bio-Rad, linear, p*I* 4–7) and rehydrated for 14 h. The absorbed protein inside the IPG strips was isoelectrically focused for a total of 14 000 Vh

Correspondence: Professor Vasanthakumari Neela, Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, 43400, Serdang, Malaysia
E-mail: neela@medic.upm.edu.my
Fax: +6-3-89413802

Abbreviation: CCB, colloidal coomassie blue

