Journal of Bioscience and Bioengineering
VOL. 117 No. 6, 769–774, 2014

ELSEVIER

www.elsevier.com/locate/jbiosc

Simple and cost effective apparatus for silver staining of polyacrylamide gels with sequential reagents addition and real time monitoring

Praveen Maurye,* Arpita Basu, and Angshuman Gupta

Fishery Resource and Environment Management Division, Central Inland Fisheries Research Institute (I.C.A.R.), Barrackpore, Kolkata, West Bengal 700 120, India

Received 5 September 2013; accepted 5 November 2013
Available online 31 December 2013

Highly reproducible results in molecular biology depend a lot on effective staining and destaining methods. Silver staining of polyacrylamide DNA and protein gel has been adopted widely in the molecular biology laboratories for detecting a very low nanogram range of sample. An efficient staining of a polyacrylamide gel requires a number of well controlled and highly sensitive steps that often becomes tiresome when done manually or when there are a number of gels to be stained simultaneously. Since, silver staining is a multistep procedure that requires proper fixation and exchange of substance, a reliable protocol is necessary and a simple apparatus may be an added advantage to carry out the steps with ease and safety. Here, we describe a simple and cost effective device made from off-the-shelf components for some established silver staining protocols. Staining is done on a tray while six graduated bottles with a liquid delivery stopcock each, is connected to the tray through silicon tubing. The used up solution is drained off completely from the staining tray through a liquid outlet stopcock using vacuum pressure. The system is fixed with a camera connected to a computer for effective control of the staining process in each step. The apparatus provides the researchers with efficient staining and real time monitoring of gels without the need for handling toxic chemicals.

[**Key words:** Protein; DNA; Polyacrylamide gel electrophoresis; Silver staining; Staining apparatus]

Several different forms of gel electrophoresis have come up as a very crucial and indispensable technique of the molecular biology laboratories in the recent decades. Polyacrylamide gel electrophoresis is commonly used for routine analysis of macromolecules at a higher resolution. Polyacrylamide gel electrophoresis is a powerful and reliable analytical technique which is extensively used in biochemistry for analyzing proteins and nucleic acids in complex samples by majority of researchers despite of recent advances (1–3). Highly reproducible results depend a lot upon the staining method adopted after electrophoresis is complete. The selection of the appropriate detection method is very important, as genomics and proteomics approaches measure quantitative changes at expression levels in biological samples. A number of different techniques exist. Ideally the detection limit should be as low as possible with an optimal signal to noise ratio. For proper quantification of proteins and nucleic acids in typical biological samples, the detection method should have a wide dynamic range and linear relationship between the quantity of biomolecules and the staining intensity. The procedure should be easy and fast to perform, non-toxic, environment-friendly and less expensive. At present, no such detection technique exists that meets all these demands. It is, therefore, necessary to choose the method which is most favorable with respect to the biological sample type and other methodical requirements. Various staining methods, in single or in combinations, have been developed for visualizing biologically active macromolecules following gel electrophoresis, using variants of Coomassie Brilliant Blue dye, silver stain, ethidium bromide, fluorescence and radioactive compounds. The Coomassie Brilliant Blue dye is mostly applied for proteins and ethidium bromide for nucleic acids due to their ease of use and low cost. However, the main limitation associated with Coomassie staining methods is its poor detection sensitivity of low quantity of protein. Labeling of proteins with radioactive isotopes and fluorescent staining, on the other hand, are simple, sensitive and mass spectrometry (MS) compatible but are generally used less frequently as compared to Coomassie staining due to the requirement of sophisticated instrumentation and expensive software. Among the various protein/DNA detection methods of polyacrylamide gels, silver staining has gained wide acceptance and popularity because of its high sensitivity, convenience, use of non-radioactive chemicals and moreover, it does not require any complicated and expensive hardware or software for the readout (4,5). Furthermore, silver staining, or at least some of its variants, is also compatible with downstream processing such as mass spectrometry (6,7). The rationale of silver staining is quite simple. Protein/DNA samples bind silver ions, which can be reduced under appropriate conditions to build up a visible image made of finely divided silver metal. However, silver staining can be a tricky business, as many artifacts and pitfalls exist. The standard silver staining protocols, in particular, comprises of mainly six different processing steps that require accurate timing: fixation, sensitization and rinses, silver impregnation and rinses, development with

* Corresponding author. Tel.: +91 9832943294, +91 33 2592 1190; fax: +91 33 2592 0388.
E-mail addresses: maurye_p@mailcity.com, maurye_p@yahoo.com (P. Maurye).

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2013.11.007

rinses and stop to end the reaction process. Since, it is a multistep procedure a number of variations can be introduced at each of these steps and thus several dozens of protocols are described in the literature, which can be somewhat confusing. Automations in silver staining process have been described by many researchers (8,9). Standard protocols are available in many reference books and staining kits are supplied by many leading manufacturers (10,11). It is, therefore, the purpose of this article to exemplify selected established protocols that are routinely followed in the laboratories. Staining of gels involves many manual processing steps (12–15) which require a very careful handling of the gel, as gels are very fragile by nature and each step should be done very patiently to prevent any loss of precious results. Here, we describe a simple system for performing such protocols with ease and minimum handling of the gel and toxic chemicals. Though several commercial systems for automating certain gel staining protocols are available, these systems are much more expensive than the one described here and are less flexible. Use of this device reduces the random handling of the toxic chemicals and the results obtained are comparable with those obtained manually. The power of the instrument has been expanded considerably with the installment of a video camera that enables one to capture images at different intervals of the development process. Thus, the apparatus allows direct visualization and monitoring of the gel being stained without interfering with the staining process. It is an apparatus in which sequential addition of the reagents and solutions can be done in a very controlled manner. The system works equally well with several gels to be stained simultaneously and thus reducing the burden on the researcher.

MATERIALS AND METHODS

Acrylamide, bis, tris base, ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), tthylenediaminetetraacetic acid (EDTA), silver nitrate, sodium thiosulfate, urea, glycerol, formaldehyde, bromophenol blue, xylene cyanol FF, sodium carbonate, Coomassie Brilliant Blue R (CBBR), methanol (MeOH), ethanol (EtOH), acetic acid (HAc) and other electrophoresis chemicals were from Sigma–Aldrich (St. Louis, MO, USA). MiniVE (8 × 9 cm gel size) and EPS600 were from Amersham Biosciences. Orbital shaker (max. 20 rpm) and optic web camera (image resolution 4608 × 3456; focus range 5 cm to infinity; manual/automatic exposure control) were from local company. Vacuum system with minimum pressure of 12″ Hg (410 mbar), vacuum tubing and Millex-FA_{50} filter were from Millipore. Vertical chamber (dimensions: L × W × H: 45 × 20 × 60 cm) and horizontal chamber (dimensions: L × W × H: 45 × 45 × 45 cm) were locally made. Protein molecular weight marker containing the following proteins: phosphorylase b (Mr 97,000), albumin (Mr 66,000), ovalbumin (Mr 45,000), carbonic anhydrase (Mr 30,000), trypsin inhibitor (Mr 20,100) and α-lactalbumin (Mr 14,400) from Amersham Biosciences and GeneRuler 100 bp DNA ladder composed of individual DNA fragments: 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp were obtained from

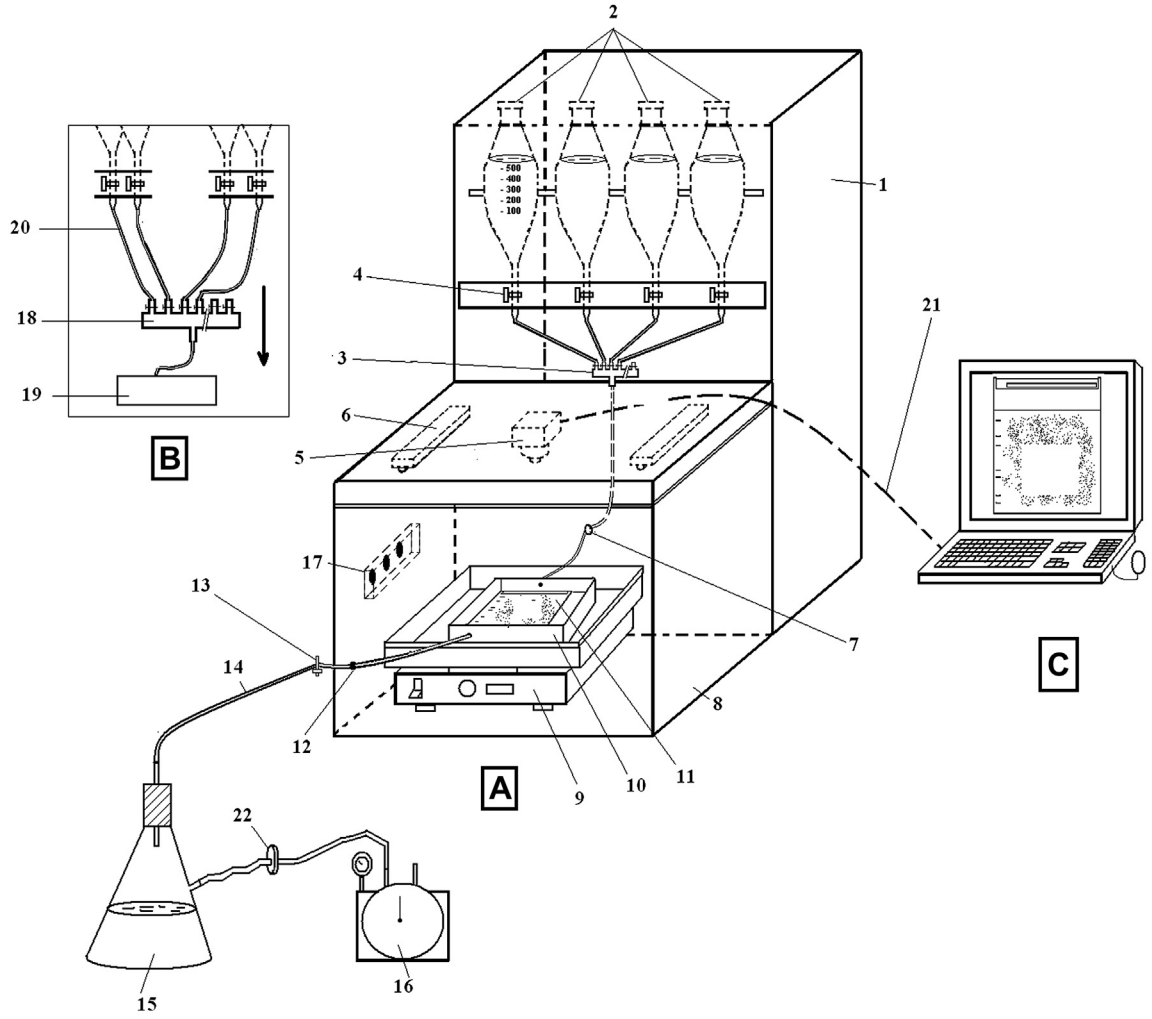

FIG. 1. Schematic diagram of the apparatus for silver staining of polyacrylamide gels. (A) Setup for the main unit: 1, vertical chamber; 2, graduated bottles; 3, delivery stopcock; 4, multi-channel stopcock; 5, camera; 6, fluorescent light source; 7, inlet port; 8, horizontal chamber; 9, shaker; 10, staining tray; 11, polyacrylamide gel; 12, outlet port; 13, outlet stopcock; 14, silicon tubing; 15, used-liquid collector; 16, vacuum pump; 17, switch board; 22, Millex filter. (B) Magnification of multi-channel stopcock with six inlet ports (no. 18) connected with graduated bottles in vertical chamber and one outlet port connected with staining tray (no. 19) by silicon tubing (no. 20) for controlled flow of solutions (arrow). (C) Computer connected with camera by computer cable (no. 21). Dimensions are not to scale. Only four graduated bottles were depicted in the main unit instead of six used.