Prevention of Solution Leakage during Polyacrylamide Gel Preparation

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Abstract: *Polyacrylamide gel electrophoresis (PAGE) is an essential technique in molecular biology. However, solution leakage often leads to the failure of PAGE gel preparation. Here a simple method is reported to prevent the leakage for mini PAGE gel preparation by filling 400 µl of 1% hot agarose solution from the bottom of the assembled glass plates. This method makes almost no solution leakage during acrylamide polymerization ensuring the successful PAGE gel preparation and no bubbles trapped beneath the bottom of the gel having to be removed before the initiation of electrophoresis.*

Keywords: *Agarose gel sealing; Polyacrylamide gel; Prevention of solution leakage*

Introduction

Polyacrylamide gel electrophoresis (PAGE) is the most common and basic experimental method for the analysis of protein or nucleic acids in molecular biology[1]. At present, the mini vertical PAGE system of Bio-Rad (CA, USA) and Tanon Science & Technology Co., Ltd. (Shanghai, China) is widely used in China. Currently, pre-casting gel of various concentrations for PAGE is available from the markets, while the technique of PAGE gel preparation is still indispensable in lab. However, solution leakage happens frequently during acrylamide polymerization after casting due to some reasons, such as the edge damage of glass plates, unsuitable glass plate assemble resulting in the failure of PAGE gel preparation.

So far, there have been some reports on prevention of solution leakage[2-5]. Zhou described the method of sealing the bottom of glass plates with 1% agar[6]. However, these methods are not applicable in actual operation due to either needing special devices or insufficient descriptions. Here we summarized a simple, rapid and applicable method to prevent solution leakage during the preparation of polyacrylamide gel.

Materials and Methods

Equipments and Materials

Mini vertical PAGE systems (Bio-Rad, CA, USA; Tanon Science & Technology Co., Ltd., Shanghai, China), 1% agarose in 378 mmol/L Tris-HCl (pH8.8) or H_2O, test-tube racks, incubator, microwave oven, 1 mL micropipette and tips.

Methods

1. Melt 1% agarose with microwave oven, and keep it as well as 1 mL pipette, tips and assembled glass plates in an incubator setting at 50°C (Fig. 1).

2. Take out assembled glass plates and inject 400 μL of 1% hot agarose solution into the space of glass plates from the bottom with 1 mL micropipette, and wag the glass plates quickly to make the agarose solution spread all over the bottom area, then stand upright onto two racks as shown in Fig. 2. After standing for 2 minutes, the agarose solution solidifies which seals the bottom space of the glass plates.

3. Fix the bottom-sealed glass plates to the frames provided by the manufacturer (Fig. 3). At this moment, it is ready for pouring the acrylamide solution mix to the space of glass plates from the top to make running gel.

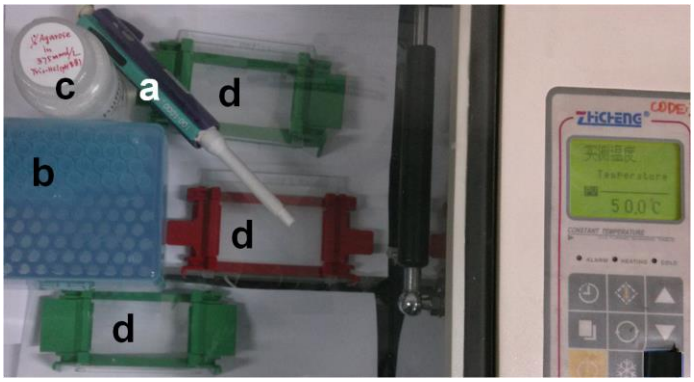

Figure 1. Pre-heating the materials for sealing the bottom of glass plates

a. 1 mL micropipette. b. 1 mL tips. c. 1% agarose. d. Assembled glass plates.

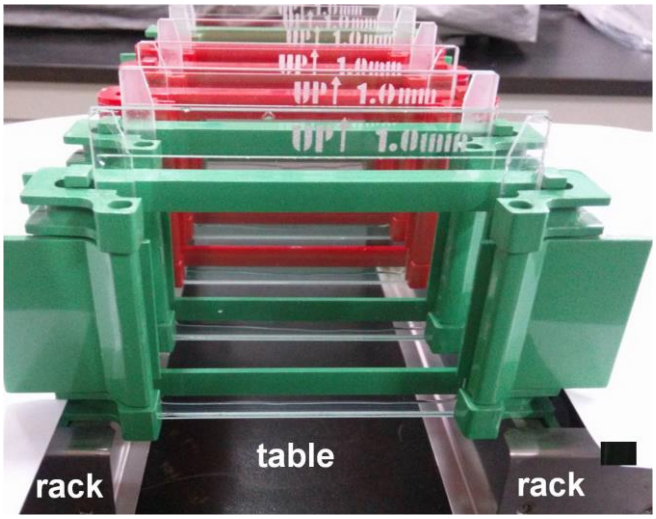

Figure 2. Placing bottom-filled glass plates on two racks

Figure 3. Fixing the bottom-sealed glass plates to the frames for casting

Precautions

During the operation, we need to pay attention to the following three points.

1. After the injection of agarose solution, the glass plates need to be shaken slightly for several times quickly, so that the agarose solution could be evenly distributed at the bottom of the whole glass plates. Slow operation might result in agarose solidification in partial region instead of the whole bottom area.

2. After the confirmation that the injected agarose solution distributes the whole bottom of the glass plates, stand them onto the two racks to prevent the bottom area touching any objects, otherwise the solution will flow out (Fig. 2).

3. Bottom-sealed glass plates must be fixed to the frames provided by manufacturer before the casting of acrylamide solution mix (Fig. 3).

Advantages

1. Simple and fast operation. At least 5 glass plates could be sealed in 1 min.

2. Remarkable sealing. This method has been used almost 3 years and accomplished by more than 10 researchers in our lab. The rate of leaking prevention has reached almost 100%.

3. No needs for specific equipments. The instruments or equipments necessary for this method are incubator, microwave oven, micropipette and tube rack, which are all routine devices for the general molecular laboratory.

4. No needs for specific chemicals. 1% agarose solution could be made with pure H_2O or 0.375 mol/L Tris-HCl (pH 8.8), the latter could be obtained by 4 times dilution of 1.5 mol/L Tris-HCl (pH 8.8), which is necessary for running gel preparation for PAGE.

5. No bubbles. Due to no space left at the bottom of the sealed glass plates, no bubbles trapped beneath the gel and therefore it is not necessary to remove the bubbles before the initiation of electrophoresis[1].

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