伯乐 BIO-RAD 垂直电泳仪简易操作指南

步骤:

制备分离胶

1. 将两块干净的玻璃板组装为电泳设备的玻璃板夹层，并将夹层固定在支架上。

2. 在 25 ml 锥形瓶中制备分离胶溶液。

3. 使用移液枪，把分离胶溶液转移到夹层之间，直到溶液的高度距离短玻璃板顶部约 2cm。

4. 用移液枪缓慢注入少量无水乙醇以消除气泡，使得凝胶形成过程中形成平面。在室温下聚合 20-30 分钟，使凝胶凝固。

制备浓缩胶

5. 倒出或用滤纸轻轻吸走分离凝胶表面的无水乙醇。

6. 制备浓缩胶，使用移液枪加在分离胶顶部，直至溢出。

7. 将带孔的梳子插入浓缩胶溶液层。

8. 室温下静置浓缩胶 20-30 分钟，使其聚合凝固。

准备样品和 1×SDS 电泳缓冲液

9. 用 5X SDS 上样缓冲液：β-巯基乙醇（99:1）配置，并加入 4 倍体积的样品样品，转移至微离心管中，并在 100°C 水浴下加热 10 分钟。

10. 将玻璃夹层从支架卸除，安装进电泳槽中，红色电极对准电泳槽中红色标签。将电泳缓冲液倒入玻璃槽中，使得浓缩胶浸泡在液体中。

11. 仔细移除梳子，避免损坏凝胶，用电泳缓冲液反复润洗凝胶加样槽。

12. 使用微量进样器在不同的孔中小心地添加 5μl 蛋白标准品和 10μl 样品。

13. 在电泳槽中加入电泳液至标线处。

跑胶

14. 将电源连接到电池。打开电源，将模式设置为 75v 恒定电流，约 30-40min；按“运行”开始电泳，直到溴酚蓝示踪染料进入分离凝胶。按“停止”以停止系统。

15.将电流增加至 110v，并运行约 1-2 小时。溴酚蓝示踪染料到达分离凝胶底部后，断开电源。

拆卸凝胶

16. 倒出电泳缓冲液，并拆卸出玻璃夹层。

17. 使用凝胶释放器轻轻打开玻璃板，然后切除浓缩胶。

用考马斯蓝染液染色凝胶蛋白

19. 将分离胶置于带盖塑料容器中，倒入过量 0.25%考马斯蓝染液，至凝胶完全浸没。

20. 轻轻摇动培养 2 小时。

21. 去除考马斯蓝染色溶液，用蒸馏水冲洗凝胶直至没有明显颜色流出。

22. 加入脱色溶液，轻轻晃动培养。

23. 30 分钟后，更换脱色溶液。

24. 轻轻晃动凝胶过夜或直到获得清晰的背景。

BIO-RAD Electrophoresis Operation Manual

Procedures:

Pour the separating gel

1. Assemble the glass-plate sandwich of the electrophoresis apparatus using two clean glass plates (used in a pair) and lock the sandwich to the casting stand.

2. Prepare the separating gel solution in a 25-ml Erlenmeyer flask.

3. Use a pipette, apply the separating gel solution in between the glass plates until the height of the solution about 2cm from the short glass plate top.

4. Use a pipette to slowly inject a small amount of absolute ethanol to eliminate bubbles. Allow a flat interface to form during gel formation. polymerize 20 to 30 min at room temperature.

Pour the stacking gel

5. Pour off or use a paper towel to gently absorb the absolute ethanol on the surface of the separation gel.

6. Prepare the stacking gel solution and add on top of separation gel until it is full.

7. Insert a comb with wells into the layer of stacking gel solution.

8. Allow the stacking gel solution to polymerize 20 to 30 min at room temperature

Prepare the sample and 1× SDS electrophoresis buffer

9. Prepare samples with SDS-loading buffer, β-Mercaptoethanol and heat 10 min at 100°C in a microcentrifuge tube.

10. Disassemble and attach gel sandwich to upper buffer chamber. The red electrode should be directed at red marker in cell. Partially fill the upper buffer chamber with electrophoresis buffer so that the sample wells of the stacking gel are filled with buffer.

11. Carefully remove the comb without tearing the edges of the polyacrylamide wells. After the comb is removed, rinse wells with electrophoresis buffer.

12. Use a micropipette to add 5µl marker and 10µl samples carefully at different wells.

13. Fill the lower buffer chamber with the amount of electrophoresis buffer

Run the gel

14. Connect the power supply to the cell. Switch on the power and set the mode to constant current at 75v with approximately 30-40min; Press “run” to start electrophoresis until the bromphenol blue tracking dye enters the separating gel. Press ”stop” to discharge the system.

15. Then, increase the current to 110v and run for approximately 1-2h. After the bromphenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Disassemble the gel

16. Discard electrode buffer and remove the upper buffer chamber with the attached gel sandwich.

17. Use the gel releaser to gently open the glass plate, remove the stacking gel.

18. Carefully remove the gel from the lower plate.

Staining protein gels with Coomassie blue

19. Place polyacrylamide gel in a plastic container with a lid containing an excess of 0.25% Coomassie blue staining solution.

20. Incubate with gentle shaking for 2h.

21. Remove the Coomassie blue staining solution and rinse the gel briefly with distilled water.

22. Add de-staining solution and incubate with gentle agitation.

23. After 30 minutes, refresh the de-staining solution.

24. Incubate the gel with gentle agitation overnight or until a clear background is obtained.