For Research Use Only. Not For Use In Diagnostic Procedures

Version 2.0

磷酸化蛋白凝胶检测试剂盒

Phosphoprotein Staining Kit

Do not eat Store at 2-8°C

Cat.No.MG1925 **Size : 10 Mini-Gels**

Technical literature is available at: www.mesgenbio.com.
E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com

Description

This kit method depends on the hydrolysis of the phosphoprotein phosphoester linkage using sodium hydroxide in the presence of calcium ions. The gel containing the newly formed insoluble calcium phosphate is then treated with ammonium molybdate in dilute nitric acid. The resultant insoluble nitrophospho-molybdate complex is stained with Methyl Green. After destaining, the phosphoproteins are colored green to green-blue. The detection limit is in the nanogram range, but depends on the degree of phosphorylation of the protein. This method will detect the phosphoproteins phosvitin and β-casein in the 40-80 ng/band and 80-160 ng/band range, respectively. The method presented here is for staining minigels. Volumes will need to be increased for larger gels.

Procedure

1. Place the gel in a dish containing 50 ml of deionized H_2O. Agitate the gel on a reciprocal or orbital shaker table for 10 minutes.
2. Move the gel to a dish containing 25 ml of **Solution 1**, and agitate the gel on a reciprocal or orbital shaker table for 15 minutes.
3. Place the gel in 25 ml of **Solution 2**, and agitate the gel on a reciprocal or orbital shaker table for 30 minutes.
4. Rinse the gel rapidly with deionized H_2O to remove the calcium chloride adhering to the surface of the gel.
5. Transfer the gel to 25 ml of **Solution 3**, cover the tray with a lid, and incubate it for 20 minutes at 65℃.
6. Place the gel in 25 ml of **Solution 4**, and agitate the gel on a reciprocal or orbital shaker table for 10 minutes.
7. Repeat Step 6.
8. Transfer the gel to 25 ml of **Solution 5**, and agitate the gel on a reciprocal or orbital shaker table for 20 minutes.
9. Place the gel in 25 ml of **Solution 6**, and agitate the gel on a reciprocal or orbital shaker table for 20 minutes.
10. To destain, transfer the gel to 25 ml of **Solution 1**, and agitate the gel on a reciprocal or orbital shaker table for 15 minutes.
11. Repeat Step 10. At this time, green spots of phosphoproteins should be seen.
12. To completely destain the gel, place it in 25 ml of **Solution 7**, and agitate the gel on a reciprocal or orbital shaker table overnight. During the destaining, change the acetic acid once, after the solution has become green in color.
13. Following visualization of the phosphoproteins, stain the total proteins on the gel using a Coomassie-Blue-staining method.

Storage condition

2-8°C

For Research Use Only. Not For Use In Diagnostic Procedures.

操作步骤（中文）

1. 请将各浓缩液用双蒸水稀释相应倍数制成 **1X 浓度工作液**再使用。
2. 将 **Solution 3** 置于 65°C 水浴中保温。
3. 取出电泳后的聚丙烯酰胺凝胶，用超纯水漂洗 10min，加入 25 mL 的 **Solution 1**，置于摇床上，振荡 15 min。
4. 倾出 **Solution 1**，加入 25 mL **Solution 2**，置于摇床上，振荡 30 min。
5. 倾出 **Solution 2**，加入 25 mL 超纯水，置于摇床上，振荡 3 min，重复此步骤 1 次。
6. 倾出超纯水，加入 25 mL **Solution 3**，置于 65°C 恒温培养箱中，静置 20 min。
7. 倾出 **Solution 3**，加入 25 mL **Solution 4**，置于摇床上，振荡 10 min。重复此步骤 1 次。
8. 倾出 **Solution 4**，加入 25 mL **Solution 5**，置于摇床上，振荡 20 min。
9. 倾出 **Solution 5**，加入 25 mL **Solution 6**，置于摇床上，振荡 20 min。
10. 倾出 **Solution 6**，加入 25 mL **Solution 1**，置于摇床上，振荡脱色 5~10 min，重复此步骤 1-2 次。
11. 待绿色的条带显示后，加入 25 mL **Solution 7**，置于摇床上，振荡过夜进一步脱色。

注意事项

1. 实验前，根据所染胶块的数量，取一定体积的各种溶液，用超纯水稀释为 1X 浓度后再使用。
2. 本试剂盒的检测灵敏度取决于目的蛋白质的磷酸化程度，由于不同磷酸化蛋白磷酸化程度不同，所以本试剂盒对于不同磷酸化蛋白的检测灵敏度也有所不同。
3. 须将装凝胶的容器和凝胶表面残留的 Ca^{2+}清洗干净，否则将导致高背景且难于脱色；但水洗时间不可过长，否则将导致检测灵敏度下降，甚至实验失败。
4. 65°C 恒温培养箱中装凝胶的容器须盖上盖子，否则可能导致凝胶卷曲，并产生高背景。
6. 本说明书所使用的试剂量和实验时间是针对 80×60×1 mm 的凝胶制订的，若凝胶体积较大或较小，可适量调整试剂用量和实验时间。
7. 产品中的保存条件及有效期均以未开封情况下计算，为了防止产品与空气接触发生化学反应影响产品性能，将未使用完毕的组分按存储要求保存同时建议开封后的组分尽快使用完毕。
8. 使用后请旋紧瓶盖，防止溶液挥发和与空气中的物质发生化学反应。

试剂盒组分

组分	体积
Solution 1 6X	125ml
Solution 2 1X	250ml
Solution 3 4X	65ml
Solution 4 10X	25ml
Solution 5 2X	125ml
Solution 6 5X	50ml
Solution 7 10X	25ml

保存条件

2-8°C，保质期两年。

产品仅供科学研究 禁止用于临床诊断