PRODUCT INFORMATION

Phosphoprotein Staining Kit
(Cat# PPS-0800)

Introduction:

At high pH environment, the phosphate group on phosphoprotein is hydrolysed into PO^{3+} ion, the calcium ion will combine the PO^{3+} ion into insoluble phosphate calcium. The phosphate calcium will form another insoluble compound after treated with ammonium molybdate and HNO_3, which can be stained by methyl green and form blue or green–blue strip on SDS-PAGE gel. The whole procedure has high sensitivity and especially for phosphoprotein, the sensitivity is decided by the phosphorylation degree of different individual phosphoprotein samples, and can detect 50 ng casein. The kit is sufficient for 20 phosphoprotein stains.

Component:

	Item	Volume			Item	Volume
1	Solution 1	6x200ml		5	Solution 5	2x200ml
2	Solution 2	1x400ml		6	Solution 6	5x80ml
3	Solution 3	4x100ml		7	Solution 7	10x40ml
4	Solution 4	10x40ml				

Note: before use, dilute the solution into 1x.

Procedures:

1. Before use, heat Solution 3 to 60°C for several minutes.
2. Transfer the SDS-PAGE gel into a container, add 20 ml of Solution 1 and place the container on a shaker, shake for 30 minutes.
3. Discard the above solution and add 20 µl of Solution 2, shake for 30 minutes.
4. Discard solution 2, add 20 ml of ddH_2O, shake for 3 minutes, and discard the ddH_2O. Repeat ddH_2O purgation procedure one more time.
5. Discard the ddH_2O, add 20 ml of Solution 3, and place the container in incubator at 60°C for 20 minutes.
6. Discard solution 3, add 20 ml of Solution 4, place the container on a shaker, and shake for 10 minutes.
7. Discard solution 4, add 20 ml of Solution 5, place the container on a shaker, and shake for 20 minutes.
8. Discard solution 5, add 20 ml of Solution 6, place the container on a shaker, and shake for 15 minutes.
9. Discard Solution 6, add 20 ml of Solution 1, place the container on a shaker, and shake for 5 minutes. Repeat this step one more time.
10. When the blue or green-blue strip appears, add 20 ml of Solution 7, place the container on a shaker, and shake for overnight.

Note:

1 At step 4, the gel and container must be fully purged, otherwise the residual Ca^{2+} will result in high background. However, prolonged purgation will decrease the sensitivity of gel staining.

2 At step 5, please avoid the gel become curly; otherwise it will have high background. Meantime, do not shake the container as it will decrease the sensitivity.

3 The kit can only be used for *in vitro* experiments.

4 The indicated volume of each solution is suitable for 80mm×60mm×1mm gel, if using a large gel, please increase the volume of solution and adjust the staining time.

Storage:

Keep the kit at 4°C, avoid light.

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