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A Geno Technology, Inc. (USA) brand name

PAGE-Perfect™

For Preparing Samples for Gel Electrophoresis

(Cat. # 786-123, 786-123T)

INTRODUCTION

Samples loaded on electrophoresis gels should ideally have identical buffer conditions and conductivity; otherwise, it may lead to distortion in the migration patterns of the protein bands**.** On the other hand, when a protein solution is dilute, it may be difficult to load an appropriate amount of protein on the gel without concentrating the protein solution first. The PAGE-Perfect™ kit has been specifically developed for preparing protein samples for loading on electrophoresis gels (Patents Pending).

The kit is based on quantitative precipitation and concentration of protein solutions, using Universal Protein Precipitation Agent (UPPA) (Patents Pending). Protein solutions as dilute as 1ng/ml can be quantitatively precipitated into a small volume. Protein precipitation is not affected by the presence of detergents, chaotropes, or other common laboratory agents. After precipitation, the precipitate is washed to remove salts and other agents which produces protein samples of conductivity ~40-60µS; ideal for critical electrophoresis. The precipitate is reconstituted in a small volume of the sample loading buffer and then loaded on electrophoresis gels for perfect protein migration patterns. If the protocol is followed correctly, the recovery is generally 100%.

The kit is suitable for processing up to 50 protein samples, 1-100µl/each.

APPLICATIONS

PAGE-Perfect™ is suitable for concentrating and preparing protein solutions for gel electrophoresis, isoelectric focusing, and 2D-gel electrophoresis.

ITEM(S) SUPPLIED

Description	**Cat. # 786-123**	**Cat. # 786-123T**
OrgoSol Buffer	50ml	5ml
PAGE-Perfect™ Buffer-I	2.0ml	2ml
PAGE-Wash	2.0ml	2ml
SDS-PAGE Sample Loading Buffer [2X]	2.5ml	0.5ml
SEED	300µl	300µl
UPPA-I	15ml	2ml
UPPA-II	15ml	2ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store all the components at room temperature upon arrival.

ADDITIONAL ITEMS REQUIRED

- Centrifuge,
- Centrifuge Tubes
- Microfuge
- β-mercaptoethanol

PREPARATION BEFORE USE

For maintaining high reducing capacity, each day add fresh β -mercaptoethanol into Sample Loading Buffer (10µl β -mercaptoethanol per 100µl PAGE-Sample Buffer). For running non-reducing gels, β -mercaptoethanol should not be added in PAGE-Sample Buffer.

NOTE: *Store OrgoSol Buffer at –20º C for ~1hr or more before use- see step 8 of the protocol.*

IMPORTANT NOTES

- Perform the entire procedure at 4-5°C (ice bucket) unless specified otherwise. Various incubation conditions must be strictly followed. Use 1.5ml microfuge tubes for processing protein samples. 0.5ml microfuge tubes are not recommended.
- Always position the microfuge-tubes in the centrifuge in the same orientation, i.e. cap-hinge facing outward. This will allow the pellet to remain glued to the same side of the tube during centrifugations and washing steps and minimize the loss of the protein pellets.

PROTOCOLS

1. Transfer 1-100µl protein solution (containing 1-100µg protein per sample) into a 1.5ml microfuge tube.
2. Add 300µl UPPA-I and mix well. Incubate at 4-5^{o}C (ice-bucket) for 10-15 minutes.
3. Add 300µl UPPA-II in to the mixture of protein and UPPA-I, then vortex the tube.
   ***NOTE**: For larger sample size, use 3 volumes each of UPPA-I and UPPA-II for each volume of sample.*
   ***NOTE**: Also read the modification below, Processing Large Samples.*
4. Centrifuge the tube at 15,000xg for 5 minutes to form a tight protein pellet.
5. As soon as the centrifuge stops, remove the tube from the centrifuge.
   ***NOTE**: Pellets should not be allowed to diffuse after centrifugation is complete.*
6. Carefully and without disturbing the pellet, use a pipette tip to remove & discard the entire supernatant.
7. Carefully re-position the tube in the centrifuge as before, i.e. cap-hinge facing outward. Centrifuge the tube again for 30 seconds. Use a pipette tip and remove the remaining supernatant.
8. Add 40µl of PAGE-Wash on top of the pellet. For larger sample size, add a Wash volume 3-4 times the size of the pellet.
9. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing outward. Centrifuge the tube again for 5 minutes. Use a pipette tip, remove and discard the Wash.
10. Add 25µl of pure water on top of the pellet. For large sample size, add enough water to just cover the pellet, i.e. a volume equal to the size of the pellet. Vortex the tube.
    ***NOTE**: Pellets do not dissolve in water.*
11. Add 1ml OrgoSol Buffer, pre-chilled at -20°C, and 5µl SEED. For a large sample size, add 1ml OrgoSol Buffer for each 0.1-0.3ml protein solution. In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Step 10.
12. Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer.
    ***NOTE**: Pellets do not dissolve in water.*
13. Incubate the tube at -20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.
14. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.
15. Remove and discard the supernatant. You will notice a white pellet in the tube. Air-dry the pellet. On drying, the white pellet will turn translucent.
    ***NOTE**: Do not over dry the pellet- parched dry pellets may be difficult to dissolve.*
16. Suspend the pellet in an appropriate volume of PAGE-Perfect™ Buffer-I (5-40µl PAGE-Perfect™ Buffer-I). Vortex to suspend the pellet. Incubate for 5 minutes.
    ***NOTE**: If proteolytic damage to the protein is a concern, protease inhibitor cocktail may be added at this stage.*

17. Add an equal volume (5-40µl) of PAGE-Sample Buffer containing β -mercaptoethanol (or without β -mercaptoethanol for non-reducing gels). If solution turns yellowish, add PAGE-Perfect™ Buffer-I in an increment of 0.5µl each until the solution turns blue.
    NOTE: For running non-denaturing gels, add an equal volume of an appropriate [2X] non-denaturing sample buffer.
18. Vortex and incubate at room temperature for 5-10 minutes to completely dissolve the protein pellet. Place the sample tube in a boiling water bath for 5 minutes.
19. After the boiling is complete, vortex and centrifuge the tube for 30 seconds. The sample is now ready for loading on gels. Vortex the tube before loading the protein solution on the gel.

PROCESSING LARGE SAMPLES

Samples containing > 100µg protein produces large and tightly packed protein pellets which require a longer time to dissolve in Buffers. Grinding of the protein pellet with a pestle will accelerate solubilization of the pellet. We recommend use of microfuge tubes and tight fitting pestles for processing samples containing larger than 100µg protein.

CITATIONS

1. Devillard, E. et al (2004) J. Bacteriol. 186: 136
2. Rincon, M. et al (2004) J. Bacteriol. 186: 2576
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4. Kovacina, K. et al (2003) JBC. 278: 10189
5. Grimaldi, M. et al (2003) J. Neurosci. 23: 4737
6. Wu, X. et al (2002) JBC. 277: 13597

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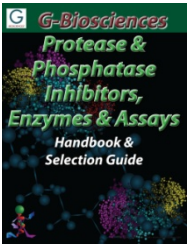

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