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A Geno Technology, Inc. (USA) brand name

PerfectFOCUS™

For Preparing Low Conductivity Samples for IEF/2D-Gel Electrophoresis

(Cat. # 786-124, 786-124T)

INTRODUCTION

Protein samples loaded on isoelectric focusing (IEF) gels should ideally have low conductivity and be free from agents known to interfere with net protein charge. These agents include ionic detergents, salts, lipids, charged polysaccharides, peptides, nucleic acids, enzyme substrates, inhibitors, plant products (phenols etc.) and agents having a charge. On the other hand, when a protein solution is dilute, it may be difficult to load an appropriate amount of the protein on the gel without concentrating the protein solution first.

The PerfectFOCUS™ kit has been specifically developed for preparing lower conductivity protein samples for isoelectric focusing gels. PerfectFOCUS™ concentrates the protein solution and removes agents such as detergents, salts, peptides, nucleic acids, lipids, phenols, and other small molecules with a charge (Patents Pending). The kit is based on quantitative precipitation and concentration of protein solutions using Universal Protein Precipitation Agent (UPPA) (Patents Pending).

Protein solution as dilute as 1ng/ml, can be quantitatively precipitated into a small volume. Protein precipitation is not affected by the presence of detergents, chaotropic, or other common laboratory agents. After precipitation, the precipitate is washed to remove salts and other agents - which produces protein sample of conductivity ~40-50µS - ideal for critical IEF/2D studies. The protein 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%.

APPLICATIONS

The PerfectFOCUS™ kit is suitable for concentrating and preparing protein solutions for isoelectric focusing (IEF) and 2D-gel electrophoresis. The regular size kit is suitable for processing up to 50 protein samples and the trial size for 6 samples, 1-100µl/each.

ITEM(S) SUPPLIED (Cat. # 786-124)

Description	Cat. # 786-124	Cat. # 786-124T
UPPA™ -I	15ml	2ml
UPPA™ -II	15ml	2ml
FOCUS™-Wash	2ml	2ml
OrgoSol Buffer™	50ml	5ml
SEED™	300µl	300µl
PerfectFOCUS™ Buffer-I	2ml	2ml
PerfectFOCUS™ Buffer-II	0.5ml	0.5ml

STORAGE CONDITIONS

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

NOTE: Chill OrgoSol Buffer at –20°C for ~1hr or more before use

ADDITIONAL ITEMS REQUIRED

- Centrifuge, Centrifuge Tubes, Microfuge

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 centrifugation and washing steps and minimize the loss of the protein pellets.

PROTOCOL

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°C (ice-bucket) for 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. See Appendix: Processing Large Samples.*
4. Centrifuge the tube at 15,000x g 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, without disturbing the pellet, use a pipette tip to remove & discard the entire supernatant.
7. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward. Centrifuge the tube again for 30 seconds. Use a pipette tip to remove the remaining supernatant.
8. Add 40µl of FOCUS-Wash on top of the pellet. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward.
   ***NOTE:** For larger sample size, add Wash 3-4 x times the size of the pellet.*
9. Centrifuge the tube again for 5 minutes. Use a pipette tip to remove and discard the Wash.
10. Add 25µl of pure water on top of the pellet.
    ***NOTE:** For large sample size, add water just enough to cover the pellet, i.e. a volume equal to the size of the pellet.*
11. Vortex the tube.
    ***NOTE:** Pellets do not dissolve in water*.
12. Add 1ml OrgoSol Buffer, pre-chilled at –20°C, and 5µl SEED.
    ***NOTE:** For large samples size, for each 0.1-0.3ml protein solution add 1ml OrgoSol Buffer. In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Step 10.*
13. Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer.
    ***NOTE:** Pellets do not dissolve in OrgoSol Buffer.*
14. Incubate the tube at –20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.
15. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.
16. 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 pellets - parched dry pellets may be difficult to dissolve*.
17. Add an appropriate volume of isoelectric focusing (IEF) loading buffer to suspend the pellet (preferably, containing 8-9M urea, detergents, ampholytes. etc.). Vortex the tube for 30 seconds. Incubate and vortex periodically until pellet is dissolved. Centrifuge and collect a clear protein solution and load on IEF gel.

Alternative Protein Solubilization Protocol

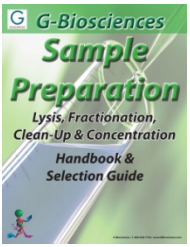
1. Add 5-40µl PerfectFOCUS Buffer-I to the pellet and vortex.
2. Incubate at room temperature for 5 minutes.
3. Add PerfectFOCUS Buffer-II and vortex for 30 seconds For each 5µl PerfectFOCUS Buffer-I used, add 1µl of PerfectFOCUS Buffer-II.
4. Vortex and incubate at room temperature for 5 minutes to completely dissolve the protein pellet. The protein solution at this stage contains 60mM Tris, pH 7-7.5.

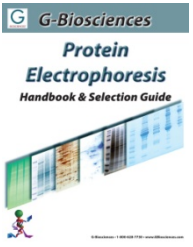
APPENDIX: PROCESSING LARGE SAMPLES:

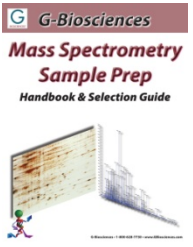
Samples containing > 100µg protein produces large and tightly packed protein pellets, which require a longer time to dissolve in Buffers. Grinding of the protein pellet with a pestle will accelerate solubilization of the pellet. We recommend use of microfuge tubes and tight fitting pestle for processing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

RELATED PRODUCTS

Download our Sample Preparation, Protein Electrophoresis and Mass Spectrometry Sample Preparation Handbooks.







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