Perfect- FOCUS™

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

INTRODUCTION

Protein samples loaded on iso-electric 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 *Perfect*-FOCUS™ kit has been specifically developed for preparing lower conductivity protein samples for iso-electric focusing gels. *Perfect*-FOCUS™ 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 *Perfect*-FOCUS™ kit is suitable for concentrating and preparing protein solutions for iso-electric 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
UPPA™ -I	15ml
UPPA™ -II	15ml
FOCUS™-Wash	2.0 ml
OrgoSol Buffer™	50 ml
SEED™	300µl
Perfect-FOCUS™ Buffer-I	2.0 ml
Perfect-FOCUS™ Buffer-II	0.5 ml

STORAGE CONDITIONS

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

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

ITEMS NEEDED BUT NOT SUPPLIED

- Centrifuge, Centrifuge Tubes, Microfuge
- Important Notes
  - I. Perform the entire procedure at 4-5º 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.
  - II. 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 1-100 µl protein solution (containing 1-100µg protein per sample) into a 1.5 ml microfuge tube. Add 300µl UPPA-I and mix well. Incubate at 4-5° C (ice-bucket) for 15 minutes.

   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]. Also read the modification below - PROCESSING LARGE SAMPLES.*
2. Centrifuge the tube at 15,000x g for 5 minutes to form a tight protein pellet.
3. As soon as the centrifuge stops, remove the tube from the centrifuge. *(**NOTE**: Pellets should not be allowed to diffuse after centrifugation is complete).*
4. Carefully and without disturbing the pellet, use a pipette tip to remove & discard the entire supernatant.
5. Carefully re-position 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.
6. Add 40 µl of FOCUS-Wash on top of the pellet (for larger sample size, add Wash 3-4 x times the size of the pellet). Carefully re-position the tube in the centrifuge as before, i.e. cap-hinge facing out-ward. Centrifuge the tube again for 5 minutes. Use a pipette tip to remove and discard the Wash.
7. Add 25µl of pure water on top of the pellet (for large sample size, add water just enough to cover the pellet, i.e. a volume equal to the size of the pellet). Vortex the tube. ***Please note**, pellets do not dissolve in water*.
8. Add 1 ml OrgoSol Buffer, pre-chilled at –20º C, and 5µl SEED. [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 7].

   Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer. Please note, pellets do not dissolve in OrgoSol Buffer. Incubate the tube at –20º C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.
9. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.
10. 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*.
11. Add an appropriate volume of iso-electric 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.

Alternatively: Suspend the pellet in *Perfect*-FOCUS Buffer-I & *Perfect*-FOCUS Buffer-II as follows.
Add 5-40 µl *Perfect*-FOCUS Buffer-I on the pellet and vortex. Incubate at room temperature for 5 minutes.
Add *Perfect*-FOCUS Buffer-II and vortex for 30 seconds (for each 5 µl *Perfect*-FOCUS Buffer-I used, add 1µl of *Perfect*-FOCUS Buffer-II).
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.

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

1. ***Non-Interfering™ Protein Assay (Cat # 786-005)*** *– A protein assay that is not affected by the presence of common laboratory agents such as detergents, reducing agents, EDTA, dyes, etc.*
2. ***Spin-OUT™ (Cat # 786-171)*** *– A spin column suitable for buffer exchange or removal of small molecules from protein and nucleic acid solutions.*

***NOTE**: For other related products, visit our web site at www.GBiosciences.com or contact us.* *Rev:08.27.10/IA*