OrgoSol DETERGENT-*OUT*™ Kit

Concentrate and Remove Detergents From Protein Solution

INTRODUCTION

The column-based method of removing detergents is simple to use. These detergent removing columns have limited binding capacity and are not suitable for hydrophobic proteins. In addition, for ionic and non-ionic detergents two different types of detergent removing columns are needed. The *OrgoSol* Detergent-*OUT*™ kit has been specifically developed for removing all types of detergent from protein solution. The *OrgoSol* Detergent-*OUT*™ can be used for removing ionic, non-ionic and cationic detergents and also suitable for removing detergents from hydrophobic proteins. This kit is based on protein precipitation, followed by removal of detergent from the protein pellet. After removing detergent, the protein pellet is suspended in buffer of choice.

The *OrgoSol* DETERGENT-*OUT*™ kit is supplied in two sizes: the **Micro** Kit is for removing detergents from up to a total of 10ml dilute protein solution, either single or multiple procedures, and the **Medi** Kit is for removing detergents from up to a total of 30 ml dilute protein solution, either single or multiple procedures. Additional volumes of any reagent may be purchased separately.

APPLICATIONS

Removal and concentration of protein solutions for iso-electric focusing, 2D gels, raising antibodies, electrophoresis, protein assays, and other applications.

ITEMS SUPPLIED	Cat # 786-127 (Micro)	Cat # 786-128 (Medi)
UPPA-I	30ml	100ml
UPPA-II	30ml	100ml
OrgoSol Buffer	50ml	2 x 50ml
DO Wash	2.0ml	2 x 2.0ml
SEED	300µl	2 x 300µl
D O Prep Buffer – I	2.0ml	2 x 2.0ml
D O Prep Buffer – II	0.5ml	2 x 0.5ml

ITEMS NEEDED BUT NOT SUPPLIED: Centrifuge, Centrifuge Tubes, Microfuge, & Spin Columns

STORAGE CONDITION

Shipped at ambient temperature. Store all the components at room temperature upon arrival.
Note: *Store OrgoSol Buffer at –20° C for ~1hr or more before use- see step 9 of the protocol.*

PROTOCOLS

Important Notes

Perform the entire procedure in the cold (ice bucket) unless specified otherwise. Concentration should be performed in a centrifuge tube. For small volumes, use microfuge tubes. Always position microfuge-tubes in the centrifuge at the same orientation, i.e. cap-hinge facing out-ward. This will allow the pellet to remain glued to the same side of the tube during repeated centrifugations and minimize the loss of protein pellets.

1. Mix 1 volume of protein solution with 3 volumes of UPPA-I (See Example below). Vortex the mixture and incubate at 4-5^{0}C (ice bucket) for 10 minutes.

2. Add 3 volumes of UPPA-II in to the mixture of protein and UPPA-I (See Example below). Vortex and centrifuge the tube.

Example: For 0.1ml protein solution, add 0.3ml UPPA-I, incubate and then add 0.3ml UPPA-II. Also, read modifications below- PROCESSING LARGE SAMPLES.

3. Centrifuge the tube at 15,000xg for 5 minutes to form a tight pellet.

4. As soon as the centrifuge stops, remove the tube from the centrifuge. (NOTE: Pellets should not be allowed to diffuse after centrifugation is complete).

5. Carefully and without disturbing the pellet, remove the entire supernatant.

6. 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 pipet tip and remove the remaining supernatant.

7. Add 40µl of DO Wash on top of the pellet (for large sample size, add DO 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 pipet tip, remove and discard the wash.

8. Add 25µl of pure water on top of the pellet (i.e., add water just enough to cover the pellet - a volume equal to the size of the pellet). Vortex the tube. Please note, pellets do not dissolve in water.

9. For each 0.1ml-0.3ml protein solution add 1ml OrgoSol Buffer (***pre-chilled at -20^{O}C***), and 5µl SEED.
[In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Step 8].

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 –20^{o}C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.

10. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.

11. 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.

12. Suspend the pellet in an appropriate volume of DO Prep **Buffer-I** (5-50µl DO Prep **Buffer-I**). Vortex to suspend the pellet. Incubate for 5minutes.

13. Add DO Prep **Buffer-II**. For each 5µl DO Prep **Buffer-I** used, add 1µl of DO Prep **Buffer-II**. Incubate for 5 minutes. After the pellet is dissolved, centrifuge and collect a clear protein solution. The protein solution at this stage contains 60mM Tris, pH 7-8.

After dissolving the pellet, the protein solution may be mixed with Urea, GunHCl, SDS-PAGE gel loading buffer or other types of buffers and agents.
For buffer exchange, the protein suspension may be dialyzed or passed through a pre-equilibrated spin column.

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 more than 100µg protein. SEE RELATED PRODUCTS FOR ORDERING.

RELATED PRODUCTS

1. ***Pestles & Tubes (Cat # 786-138P)*:** For grinding small samples of tissue etc.
2. ***Non-Interfering Protein Assay***™**:** A protein assay that is not affected by the presence of common laboratory agents such as detergents, reducing agents, EDTA, dyes etc.
3. ***Detergent-OUT***™*:* For the removal of SDS, Triton-X100 and other detergents from protein solutions.
4. ***Tube-O-DIALYZER***™: For the dialysis of small samples.
4. ***Spin-OUT Columns*:** For buffer exchange or removal of small molecules from protein and nucleic acid solutions

For additional information, please visit our web site www.GBiscience.com or contact us.