CHEMICON® INTERNATIONAL
a Serologicals® Company

PROTEIN-CONCENTRATE KIT (MICRO)

Concentrate Dilute Protein Solutions

CATALOG NUMBER: 2100

LOT NUMBER:

QUANTITY: 1 Kit

DESCRIPTION: The Chemicon Protein-Concentrate kit uses a proprietary reagent Universal Protein Precipitation Agent (UPPA). Protein solutions as dilute as 1 ng/mL can be quantitatively concentrated into a small volume. Protein precipitation is not effected by the presence of detergents, chaotropic, or other common laboratory agents. After precipitation, the precipitate is suspended in a small volume of Precipitate Suspension Buffer supplied with each kit. If the protocol is followed correctly, the recovery is generally 100%.

There are enough reagents for concentrating up to 10 mL of dilute protein solution.

APPLICATIONS: Suitable for concentrating proteins for running gels, raising antibodies, protein purification, protein assays and other applications.

This kit is not suitable for those proteins which may lose some of their biological activities when precipitated.

KIT COMPONENTS:

UPPA-I	30 mL
UPPA-II	30 mL
OrgoSol Buffer	50 mL
UPC-Wash	2.0 mL
UPC-SEED	0.3 mL
Precipitate Buffer I	2.0 mL
Precipitate Buffer II	0.5 mL

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Centrifuge
- Centrifuge tubes
- Microfuge
- Spin columns

PROTOCOL:

Important

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 to be concentrated with 3 volumes of UPPA-I. Vortex well and incubate at 4-5°C (ice bucket) for 10-15 min.
2. Add 3 volumes of UPPA-II. Vortex and the tube.
   Example: For 0.1 mL of protein solution, add 0.3 mL of UPPA-I, incubate and then add 0.3 mL of UPPA-II.
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. Using a pipet tip, carefully and without disturbing the pellet, remove the entire supernatant.
6. 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 pipet tip and remove the remaining supernatant.
7. Add 40 μL of UPC-Wash on top of the pellet. Carefully reposition the tube in the centrifuge as before, i.e. cap-hinge facing out-ward. Centrifuge the tube again for 5 minutes. Using a pipet tip, carefully and without disturbing the pellet, remove the Wash solution.
8. Add 25 μL of pure water on top of the pellet (i.e. add just enough water to cover the pellet – a volume equal to the size of the pellet). Vortex the tube to suspend the pellet. Please note: pellets do not dissolve in water.
9. Add 1-5 mL of OrgoSol Buffer, pre-chilled at -20°C, and 5 μL of UPC-SEED. NOTE: for each 0.1-0.3 mL of protein solution add 1 mL OrgoSol Buffer. 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 will not dissolve in OrgoSol Buffer.

   Incubate the tube at -20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each time.
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 pellets – parched pellets may be difficult to dissolve.
12. Suspend the pellet in an appropriate volume of Precipitate Buffer-I (5-50 μL Precipitate Buffer-I). Vortex to suspend the pellet. Incubate for 2 minutes.
13. Add Precipitate Buffer-II. For each 5 μL Precipitate Buffer-I used, add 1 μL of Precipitate 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 60 mM Tris, pH 7-7.5.

    After dissolving the pellet, the protein suspension may be mixed with SDS, 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 > 100 μg protein produce 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.

STORAGE/HANDLING: Store at room temperature for up to 6 months from date of purchase. To avoid possible microbial contamination, dispense all solutions aseptically. The OrgoSol buffer may be stored at -20°C for up to 6 months after date of receipt.

For research use only; not for use as a diagnostic.