UPPA-PROTEIN Concentrate™

G-Biosciences

Abstract

The UPPA-PROTEIN Concentrate™ kit is used to quantitatively concentrate dilute protein samples as low as 1ng/ml into a small volume. Protein precipitation and concentration are not affected by the presence of detergents, chaotropics, or other common laboratory agents.

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Guidelines

INTRODUCTION

PROTEIN-Concentrate™ kit uses a proprietary reagent, Universal Protein Precipitation Agent (UPPA; Patent Pending). Protein solutions as dilute as 1ng/ml can be quantitatively concentrated into a small volume. Protein precipitation is not affected by the presence of detergents, chaotropics, 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 analysis. The protein precipitate is suspended in a small volume buffer. If the protocol is followed correctly, the recovery is generally 100%.

The PROTEIN-Concentrate™ kit is supplied in two sizes: the Micro Kit is for concentrating up to a total of 10 ml dilute protein solution, either single or multiple procedures, and the Medi Kit is for concentration 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

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, for such proteins use either Column-PROTEIN Concentration™ kit (Cat. #786-126) or OrgoSol PROTEIN Concentration™ kit (Cat. # 786-125).

ITEM(S) SUPPLIED

Cat. #	786-120 (Micro)	786-121 (Medi)
UPPA - I	30ml	100ml
UPPA - II	30ml	100ml
OrgoSol Buffer	50ml	2 x 50ml
UPC-Wash	2.0ml	2 x 2.0ml
SEED	300µl	2 x 300µl
Solubilization Buffer-I	2.0ml	2 x 2.0ml
Solubilization Buffer-II	0.5ml	2 x 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- see step 10 of the protocol.

ADDITIONAL ITEMS REQUIRED

Centrifuge, Centrifuge Tubes, Microfuge, & Spin Columns

NOTE: 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.

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.

Before start

Chill OrgoSol Buffer at –20°C for ~1hr or more before use- see step 10 of the protocol.

NOTE: 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.

Materials

UPPA-PROTEIN-Concentrate™ 786-120 by G-Biosciences

Protocol

Step 1.

Mix 1 volume of protein solution with 3 volumes of UPPA-I (See example in Step 3).

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Step 2.

Vortex the mixture and incubate at 4°C for 10-15 minutes.

DURATION
00:10:00

Step 3.

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

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Example: For 0.1ml protein solution, add 0.3ml UPPA-I, incubate and then add 0.3ml UPPA-II. Also, review Processing large Samples Protocol in the Guidelines.

Step 4.

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

DURATION
00:05:00

Step 5.

As soon as the centrifuge stops, remove the tube from the centrifuge.

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NOTE: Pellets should not be allowed to diffuse after centrifugation is complete.

Step 6.

Carefully and without disturbing the pellet, use a pipette tip and remove the entire supernatant.

Step 7.

Carefully re-position the tube in the centrifuge as before, i.e. cap-hinge facing outward.

Step 8.

Centrifuge the tube again for 30 seconds.

DURATION
00:00:30

Step 9.

Use a pipette tip and remove & discard the remaining supernatant.

Step 10.

Add 40µl of UPC-Wash on top of the pellet (for larger sample size, add Wash 3-4 x times the size of the pellet).

Step 11.

Carefully re-position the tube in the centrifuge as before, i.e. cap-hinge facing outward.

Step 12.

Centrifuge the tube again for 5 minutes.

DURATION
00:05:00

Step 13.

Use a pipette tip, remove and discard the Wash.

Step 14.

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).

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NOTE: Pellets do not dissolve in water

Step 15.

Vortex the tube.

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NOTE: Pellets do not dissolve in water

Step 16.

For each 0.1-0.3ml protein solution, add 1ml OrgoSol Buffer (pre-chilled at –20°C), and 5µl SEED.

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NOTE: In addition, OrgoSol Buffer must be at least 10 fold in excess of the water added in Steps 11-13.

Step 17.

Vortex to suspend the pellet.

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It is important that the pellet is fully suspended in OrgoSol Buffer.

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NOTE: Pellets do not dissolve in OrgoSol Buffer.

Step 18.

Incubate the tube at –20°C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.

DURATION
00:30:00

Step 19.

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

DURATION
00:05:00

Step 20.

Remove and discard the supernatant. You will notice a white pellet in the tube.

Step 21.

Air dry the pellet. On drying, the white pellet will turn translucent.

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NOTE- Do not over dry the pellets as these pellets may be difficult to dissolve.

Step 22.

Suspend the pellet in an appropriate volume of Solubilization Buffer-I (5-50 µl Solubilization Buffer-I).

Step 23.

Vortex to suspend the pellet.

Step 24.

Incubate for 2 minutes.

DURATION
00:02:00

Step 25.

Add Solubilization Buffer-II. For each 5µl Solubilization Buffer-I used, add 1µl of Solubilization Buffer-II.

Step 26.

Incubate for 5 minutes.

DURATION
00:05:00

Step 27.

After the pellet is dissolved, centrifuge and collect a clear protein solution. The protein solution at this stage contains 60mM Tris, pH 7-7.5.

Step 28.

After dissolving the pellet, the protein solution may be mixed with SDS, Urea, Guanidine.HCl, SDS-PAGE gel loading buffer or other types of buffers and agents.

Step 29.

For buffer exchange, the protein suspension may be dialyzed or passed through a pre-equilibrated spin column.