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Product Information

ProteoPrep® Protein Precipitation Kit

Catalog Number **PROTPR**
Store at room Temperature

TECHNICAL BULLETIN

Product Description

The ProteoPrep® Protein Precipitation Kit contains reagents for the precipitation of proteins from aqueous samples. This kit contains separate trichloroacetic acid (TCA) and sodium deoxycholate (DOC) reagents. TCA is a classic reagent for precipitating proteins;[1] however, it is not effective in quantitatively precipitating proteins below the 30 μg level. The use of TCA combined with DOC allows for precipitation of proteins down to the 3 μg level.

Protein solutions often contain buffers, salts, or other components, added during sample preparation, which may interfere with downstream analysis. Precipitation is a protein isolation procedure, which allows physical separation of the protein pellet from the aqueous solution. Upon dissolving the protein pellet, one may adjust the protein concentration of the sample and select a suitable buffer appropriate for analysis, such as electrophoresis, protein assay, and MALDI-MS analysis.

Reagents

The kit provides reagents sufficient to precipitate fifty 0.1 ml protein samples. All reagents are provided as ready to use solutions.

Trichloroacetic Acid, 100% (w/v) Solution (Catalog Number T6323) – One bottle containing 6 ml of 100% (w/v) [6.1 N] trichloroacetic acid (TCA) solution.

Deoxycholate, 0.2% (w/v) Solution (Catalog Number D3691) – One bottle containing 50 ml of 0.2% (w/v) sodium deoxycholate (DOC) solution.

Wash Solution (Catalog Number A5351) – One bottle containing 100 ml of 25% (v/v) acetone solution. Place bottle in an ice bath for at least 20 minutes prior to use.

Equipment Required But Not Provided

- ice bath
- 37 °C heating block or heating bath
- bench-top centrifuge (microcentrifuge)
- centrifugal concentrator (SpeedVac®)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

It is recommended to store the reagents at room temperature. The reagents should be stable for at least one year.

Procedures

Precipitation Procedure for Samples with Protein Levels down to 3 μg

This procedure, combining the use of TCA with DOC, allows for quantitatively precipitating proteins from samples with protein levels down to 3 μg, or from samples with unknown protein content. If precipitating protein from a sample with low ionic strength, it is suggested to increase the ionic strength to ≥10 mM with NaCl prior to the precipitation.

1. Bring the volume of a 0.1 ml sample to 1 ml by adding 0.9 ml of the 0.2% Deoxycholate Solution (Catalog Number D3691). The final concentration of DOC in the mixture is 0.18%.
   Notes: For samples with a volume <0.1 ml, increase the sample volume to 0.1 ml with 10 mM NaCl or appropriate buffer and then proceed with step 1.

   This procedure may be scaled up for samples with volumes >0.1 ml containing at least 3 μg by using proportionally more DOC solution in step 1 and proportionally more TCA solution in step 3, keeping the final concentration of the mixture for DOC in the range of 0.14–0.18% and for TCA at 9%.
2. Vortex and incubate at room temperature for 10 minutes.
3. Add 100 μl of the 100% Trichloroacetic Acid Solution (Catalog Number T6323) to the mixture. The final concentration of TCA in the mixture is 9%. A fine precipitate will form in the mixture.
4. Vortex and incubate in an ice bath for at least 15 minutes.

5. Centrifuge the mixture at 15,000 × *g* for 10 minutes at room temperature.
   Note: Increase the centrifugation time proportionally for larger samples.
6. Decant the supernatant.
7. Add 1 ml of the Wash Solution (Catalog Number A5351) to the pellet. Make sure the Wash Solution is ice cold before use.
8. Vortex to disperse the pellet. Centrifuge at 15,000 × *g* for 5 minutes at room temperature.
9. Decant the supernatant.
10. Repeat steps 7 to 9 once to completely wash the pellet.
11. Thoroughly dry the pellet in a SpeedVac for 10–20 minutes to remove any residual acetone.
    Note: As an alternative to the use of a SpeedVac, air dry the pellet for ~20 minutes. However, residual solvent may be a problem with some subsequent procedures.
12. Dissolve the pellet in the appropriate buffer for subsequent analysis (see Table 1).
    Note: Residual deoxycholate will remain in the pellet and will dissolve at pH >8. Gentle heating and vortexing may be necessary to dissolve the protein pellet.

Precipitation Procedure for Samples with Protein Levels down to 30 µg

This procedure, using TCA alone, allows for quantitatively precipitating proteins from samples with known protein levels >30 µg. In this procedure, no deoxycholate is introduced into the protein sample nor is there any possibility of carryover of residual deoxycholate from the precipitated protein pellet. This procedure may be performed with samples of low ionic strength.

1. For protein samples containing >30 µg of protein, bring the sample volume to 1 ml by adding ultrapure water. Add 100 µl of the 100% Trichloroacetic Acid Solution (Catalog Number T6323) to the 1 ml sample volume. The final concentration of TCA in the mixture is 9%. The solution will be clear to cloudy.
   Note: This procedure may be scaled up for samples with volumes >1 ml containing more than 30 µg by using proportionally more TCA solution, keeping the final concentration of the mixture at 9%.
2. Vortex and incubate in an ice bath for at least 15 minutes.
3. Centrifuge the mixture at 15,000 × *g* for 10 minutes at room temperature.
4. Decant the supernatant.
   Note: If the precipitation is being performed only to prepare a more concentrated sample, proceed to step 8. If the precipitation is being performed to remove interfering substances, proceed to step 5.
5. Add 1 ml of the Wash Solution (Catalog Number A5351) to the pellet. Make sure the Wash Solution is ice cold before use.
6. Vortex to disperse the pellet. Centrifuge at 15,000 × *g* for 5 minutes at room temperature.
7. Decant the supernatant.
8. Thoroughly dry the pellet in a SpeedVac for 10–20 minutes to remove any residual solvent.
   Note: As an alternative to the use of a SpeedVac, air dry the pellet for ~20 minutes. However, residual solvent may be a problem with subsequent procedures.
9. Dissolve the pellet in the appropriate buffer for subsequent analysis (see Table 1).
   Note: Gentle heating and vortexing may be necessary to dissolve the protein pellet.

Table 1.
Suggested Buffers for Downstream Procedures

Downstream Procedure	**Suggested Buffer**
SDS Gel Electrophoresis	1× Laemmli Sample Buffer (prepared from Catalog Number S3401)
BCA/QuantiPro Protein Determination	40 mM Sodium Carbonate Buffer, pH 11.25 (prepared from Catalog Number S4132)
Isoelectric Focusing	Solubilization Reagent, such as Protein Extraction Reagent Type 4 (prepared from Catalog Number C0356)
Tryptic Digestion In Solution	100 mM Ammonium Bicarbonate Buffer, pH 8.5 (prepared from Catalog Number A6141)

It is recommended to use a bicinchoninic acid based protein assay (Catalog Number BCA1 or QPBCA) for dissolved pellets precipitated from detergent containing protein samples. The Bradford method is not recommended after precipitation from such samples. This may be due to carryover of detergent, to which the Bradford assay is susceptible.

Using this kit, samples for 2D electrophoresis could be precipitated from phosphate buffered saline or an 8 M urea solution containing 2% 2-mercaptoethanol, and then dissolved in Protein Extraction Reagent Type 4 (Catalog Number C0356) prior to electrophoresis. However, precipitation from some detergent containing solutions, such as Protein Extraction Reagent Type 4 (Catalog Number C0356) or Protein Extraction Reagent Type 3 (Catalog Number C0731), and dissolving the protein pellet in either solution, results in poor separation by 2D electrophoresis. An alternative precipitation procedure, use of 5–10 volumes of ice cold acetone, may be more useful for preparation of 2D electrophoresis samples from detergent containing solutions.

Related Products	Catalog Number
ProteoPrep Kits	
Total Extraction Sample Kit	PROTTOT
Membrane Extraction Kit	PROTMEM
Universal Extraction Kit	PROTTWO
Protein Extraction Reagent Type 4	C0356
Protein Extraction Reagent Type 1	C0481
Protein Extraction Reagent Type 2	C0606
Protein Extraction Reagent Type 3	C0731
EZBlue™ Gel Staining Reagent	G1041
ProteoSilver™ Plus Staining Kit	PROTSIL2
BCA Kit for Protein Determination (recommended for 200–1,000 µg/ml protein)	BCA1
QuantiPro™ BCA Assay Kit (recommended for 0.5–30 µg/ml protein)	QPBCA
ProteoMass™ MALDI-MS Calibration Kits	
Protein and Peptide	MSCAL1
Peptide	MSCAL2
Protein	MSCAL3
Sodium carbonate, monhydrate	S4132
Ammonium bicarbonate	A6141
Trypsin, Proteomics Grade	T6567

References

1. Bensadoun, A., and Weinstein, D., Analytical Biochemistry, **70**, 241-250 (1976).

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