NT-40840e

Removal of interfering substance from samples before protein assays

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The following protocols are recommended to remove interfering substance for protein assays.
before BC Assay #UP40840A
before Coo Assay protein quantitation) #UPF86400
- Protocol 1: Desalting by fast dialysis or ultrafiltration.
- Protocol 2: TCA Precipitation method
- Protocol 3: Acetone Precipitation method
- Protocol 4: ElectroElution

Protocol 1a: Desalting by fast dialysis

This method may dilute slightly the sample, thus a sensitive protein assay may be preferred as MicroBC assay or Coo Assay. It is generally as a relatively long procedure (to reach thorough desalting). However, for procedures speeding, dialysis for 30min to 2hours may usually lead to sufficient desalting allowing accurate protein assay. I.e. 40% Ammonium Sulfate in 100µl sample may be assayed for protein after a 2 change very short dialysis (5min then 10min in 100ml of PBS).

1. Put 10-500µl of sample in a suitable dialysis device*,
2. Dialyse against a suitable buffer (e.g. PBS)
Chose a MWCO, typically 10KDa for all proteins, or 1000Da for peptides.
Please refer to device recommendation for dialysis procedure (duration, buffer change...) *.

*Ask interbiotech@interchim.com for FastDialyser, FloatAlyser dialysis devices.

Protocol 1b: Desalting by ultrafiltration

This method is relatively expensive. It do not work well for very small proteins, nor larger or viscous samples (clugging, polarisation of UF membrane).

1. Put 10-500µl of sample in a suitable centrifugation ultrafiltration device*,
2. Centrifuge and fill again with a suitable buffer (e.g. PBS)
Chose a MWCO, typically 10KDa for all proteins, or 1000Da for peptides.
Please refer to device recommendation for dialysis procedure (duration, buffer change...) *.

*Ask interbiotech@interchim.com for ultrafiltration devices.

Protocol 2: TCA Precipitation method (before BC Assay protein quantitation)

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The TCA precipitation is the preferred precipitation method before protein assays *.
Note: a superior TCA-like reagent is PPR #R5594A.

1. Pipet 50 µl of each standard, blank, or unknown into microcentrifuge tubes.
2. Add 450 µl of deionized water.
3. Add 100 µl of the 0.15% (w/v) sodium deoxycholate solution.
4. Add 100 µl of the 72% (w/v) Trichloroacetic acid solution (TCA) and let stand 10 minutes at room temperature (RT).
5. Vortex, then centrifuge 10 minutes in a microcentrifuge at 10,000 rpm.
6. Aspirate off the supernatant, being careful not to disturb the pellet.

For best, most accurate results, continue with the rest of the protocol. However, in some cases, a single TCA precipitation will prove sufficient for some samples. In this case, skip forward to step # 11.

7. Add 100 µl of deionized water to the pellet, followed by 100 µl each of sodium deoxycholate and TCA reagent.
8. Allow the tubes to stand for 10 minutes at RT.

9. Vortex, then centrifuge for 10 minutes in a microcentrifuge at 10,000 rpm.
10. Again aspirate off the supernatant, being careful not to disturb the pellet.
11. Add 50 µl of 5% SDS (w/v) in 0.1 N NaOH solution

* Perform protein assay as usual, with BC assay, of MicroBC Assay.
For BC aAssay, Add 1 µl of BC Assay working reagent (50vol. of A + 1vol. of B). Vortex, then incubate for 30 minutes at 37° C. Measure the absorbance at 562 nm. Subtract the absorbance of the blank from all standards and samples. Plot the standards and extrapolate the concentration of the samples from the standard curve.

Protocol 3: Acetone Precipitation method (before Coo Assay protein quantitation)

Acetone precipitation is recommended for Coo Assay, but provides lower protein recovery and less accurate results than with method 5b with BC Assay. It works eventually also with BC Assay*.

1. Pipet 50 µl of each standard or sample into 1.5 ml microcentrifuge tubes in triplicate.
2. Add 200 µl of glacial (-20°C) acetone to each tube.
3. Vortex and incubate 30 minutes at -20°C.
4. Centrifuge 10 minutes at maximum speed in a microcentrifuge.
5. Pour off the supernatants and allow the acetone to evaporate from the tubes at room temperature (RT) for 30 minutes.
6. Add 50 µl of deionized water to the protein pellets and vortex.
7. Add 1ml of Coo Assay #UPF8642. Incubate 1min at room temperature. *
8. Read absorbance at 595nm (for BC Assay, at 562 nm).
   NOTE: If the interference persists, add 200 µl more of cold acetone (-20°C) to each tube following the acetone evaporation in step 5. Then, vortex and incubate samples for 15 minutes at -20°C. Centrifuge 2 minutes at maximum speed in a microcentrifuge, pour off supernatants and allow the acetone to evaporate from the tubes for 10 minutes at RT. At this point, continue with the 1X precipitation protocol in step 6.
   CAUTION: This double precipitation protocol may result in a less linear standard curve.

*For BC Assay, add 1 ml of BC Assay working solution (50 parts of reagent A and 1 part of reagent B) to each tube and vortex. Incubate 30 minutes at 37°C. Read absorbance at at 562 nm.

Protocol 4 : elution of proteins from SDS-PAGE gels

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Protein have been separated on polyacrylamide gels in either denaturing or undenaturing condition. Protein localisation might be determined with a reversible dye (Ponceau), or *in situ* label, or a control gel (border of preparative gels). Gel pieces (unstained gel) are cutted out and treated to extract proteins. Beside electro-elution technics, proteins may be recovered (with lower yield) by passive elution:

Elution Buffer: Phosphate 0.1M pH8 (proteases inhibitors may be added, as well as SDS0.1%)

1. Make a slurry of the gel pieces of interest cryshed in the elution buffer
2. Mix the slurry with the buffer for 4 hours at room temperature (or +4°C if labile protein)
3. Recover the supernatent and dialyse or concentrate.

Ask interbiotech@interchim.com for electroelution devices: GebaFlex, Electroprep

Other information

NT-Protein assays
NT-ExtractionsOfProeins&More

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