national diagnostics

ND™ Protein Precipitation Kit

National Diagnostics Protein Precipitation Kit brings down >99% of all proteins, even complex mixtures in dilute solution. Interfering salts and surfactants are left behind in the supernatant. The precipitants are removed with a rapid and gentle wash, allowing the concentrated proteins to be recovered in a small volume of whatever buffer is optimal for the next procedure.

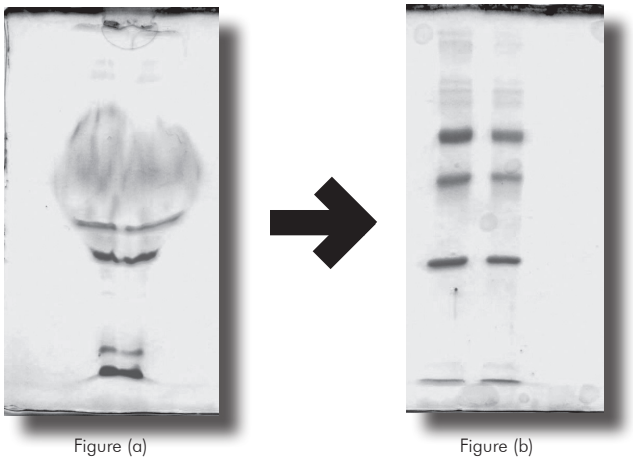


Figure (a) Figure (b)

Gels (a) before and (b) after the use of the ND Protein Precipitation Kit.

Frequently Asked Questions

Is the kit selective for membrane proteins, cytosolic proteins etc?
No. Reagent A binds non-specifically to proteins and the ratio of recovered proteins should reflect the proportion in the original solution. It is possible individual proteins precipitate with slightly different efficiencies but this has not been observed in testing.

What are the upper and lower concentration limits of protein that can be precipitated?
The lower limit for reproducible recovery of BSA is 100ng at a concentration of 0.25 µg/ml. Recovering more than 50µg of protein in a single tube is not ideal; it becomes more difficult to redissolve the protein pellet at the end of the procedure.

What MW of proteins can be precipitated?
Intact proteins between 10kD -200kD have been precipitated successfully as analyzed on SDS-PAGE gels.

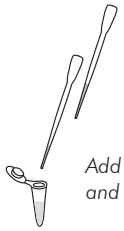
Are there special instructions for 2D electrophoresis and Mass Spectrometry?
These two procedures require the sample to be as contaminant-free as possible. The wash step is very important as it removes traces of the precipitation reagents that were used. For 2D electrophoresis and mass spec several washes may be necessary to ensure no contaminants remain with the pellet. Centrifuge after each wash.

Does the salt concentration in the sample have an effect on results?
Most salts at concentrations commonly used will not affect the precipitation method. However, very high salt concentrations, e.g. a saturated solution of NaCl (5.5M), will make it difficult to collect the pellet formed from Reagents A and B due to the high density of the solution. In this case it may be helpful to dilute the sample before starting the precipitation. Thiocyanate, iodide and perchlorate ions will cause a precipitate to form as soon as Reagent A is added and are not compatible with the kit.

Do nucleic acids co-precipitate with proteins?
Yes, to some extent. The kit cannot be used as a way to purify proteins away from nucleic acids, as some nucleic acid will co-precipitate.

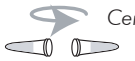
Does the pH of the starting solution affect precipitation?
The kit has been tested on protein solutions between pH 6 and pH 8 and no difference was seen in the recovery.

Protocol



Add Reagent A and Reagent B

1. Add 5 mL of Reagent A for every 100 mL of sample in a microcentrifuge tube and mix well.
2. Add 10 mL of Reagent B for every 100 mL of sample and mix.
3. Incubate for 20 minutes at room temperature, inverting the tube occasionally to promote mixing.

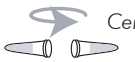


Centrifuge

4. Collect complex by centrifugation at 12,000 x g and remove supernatant. The large white pellet contains the precipitant complex and the protein.

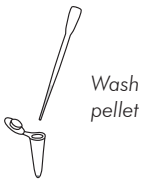
Dissolve large pellet in acetone

5. Add 1 mL acetone and mix well to ensure it completely dissolves the complex. Vortexing is generally sufficient but pipetting up and down may be necessary. There should be no clumps. Depending on the protein concentration the solution will be clear to hazy.



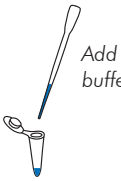
Centrifuge

6. Collect proteins by centrifugation at 12,000 x g for 10 minutes. Remove the acetone supernatant. The purified protein pellet will be small and nearly invisible for amounts less than 1 mg.



Wash small pellet

7. Wash protein pellet at least twice by suspending in 70% ethanol and collecting proteins by centrifugation. Wash at least three times for 2-D electrophoresis or mass spectrometry experiments. **NOTE:** These washes are critical to the purity of the recovered protein.



Add loading buffer

8. Air-dry pellet and dissolve in desired buffer.

ND Protein Precipitation Kit

EC-888 precipitates 50ml