national diagnostics

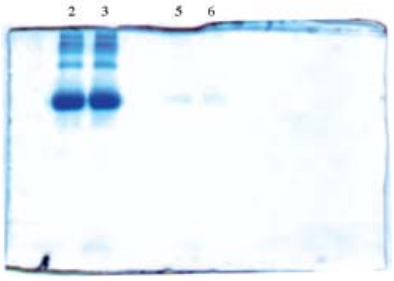
ND Protein Precipitation Kit

◆Recovery of All Proteins from Complex Mixtures
◆Precipitates as Little as 100ng of BSA at 0.25µg/ml
◆Milder than TCA
◆More Reliable than Acetonitrile or Ammonium Sulfate

Catalog number: EC-888

The ND Protein Precipitation Kit is mild on samples and easy to use. It casts the finest net of any procedure, allowing the high yield collection of all proteins in solution, precipitating even the most dilute proteins. The ND Protein Precipitation Kit allows collection of proteins that would be missed by other methods.
Ideal for both routine and difficult work, the ND Protein Precipitation Kit stands alone among all methods. It offers the best combination of mildness, simplicity and effectiveness in protein precipitation.

High Yield Recovery



10ug BSA in 1ml vol and 50 ng of BSA in 200ul were prepared from stock and precipitated following kit instructions. 10ug and 50ng of BSA in 5 ul were prepared as controls. After running on a 12% gel, lanes 2 and 3 show control and recovered 10ug BSA. Lanes 5 and 6 show control and recovered 50ng BSA. Densitometry of 10ug BSA showed 91.9% recovery.

Simplicity of Method

Reagent A and Reagent B form a coprecipitate with the protein.

After a simple wash, the protein pellet is ready to be redissolved in desired buffer.

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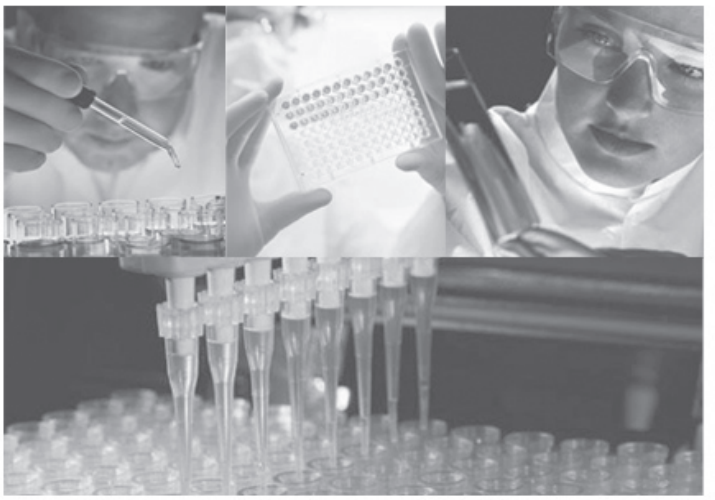

ND™ Protein Precipitation Kit

- Rapid recovery and concentration of proteins from dilute solutions
- Better than 99% recovery of all proteins from complex mixtures
- Rapid removal of contaminating salts and surfactants
- Precipitates as little as 100ng BSA @ as low as 0.25µg/ml

Introduction

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 acetonitrile or acetone wash, allowing the concentrated proteins to be recovered in a small volume of whatever buffer is optimal for the next procedure.

Protocol

1. Add 1/20 volume Reagent A to sample in a centrifuge tube and mix well.
2. Add 1/10 volume Reagent B to sample.
3. Allow to precipitate for 20 minutes at room temperature. Precipitate is comprised of Reagent A:B complex along with trapped protein molecules.
4. Collect precipitate by centrifugation and remove supernatant. The pellet will be large.
5. Completely disperse pellet in acetone to dissolve away precipitated A:B complex. The solution should appear clear to cloudy, depending on protein concentration, with no visible clumps. Undispersed clumps will trap impurities which will be carried over into the final isolate.
6. Collect proteins by centrifugation.
7. To remove salts and surfactants, wash pellet with acetone, acetonitrile or 70% ethanol. This step may be repeated if desired for heavily contaminated samples, or for downstream applications requiring the highest purity proteins. Collect proteins by brief centrifugation if necessary.
8. Redissolve pellet in desired buffer.

Frequently Asked Questions

Is the kit selective for membrane proteins, cytosolic proteins etc?
The kit is not selective for a particular class of protein. Reagent A binds non-specifically to proteins and the ratio of recovered proteins should reflect the proportion in the original solution. It is possible that 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 at 200 µg/ml may not be ideal because it becomes more difficult to redissolve the protein pellet at the end of the procedure. Above this concentration it may be helpful to divide the samples among several tubes or dilute the sample before precipitation. We are not aware of any commercially available kit which can recover below 2µg which matches the simplicity of the ND Protein Precipitation Kit, which does not involve centrifuge concentration.

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

Are there special instructions for 2D electrophoresis and Mass Spectrometry?
2D electrophoresis and mass spectrometry require the sample to be as contaminant-free as possible. The wash step is very important in this regard as it removes traces of the precipitation reagents that were used. For 2D electrophoresis and mass spec several washes (at least 2) may be necessary to ensure no contaminants remain with the pellet. Centrifuge after each wash and be careful not to dislodge the pellet.

Does the concentration of salt in the sample have an effect on the results?
Most salts at concentrations used in biological laboratories will not affect the precipitation method. However, the surrounding solution can effect kit performance in a few instances. Very high salt concentrations e.g. a saturated solution of NaCl (5.5M) will make it difficult to collect the pellet formed by adding reagent 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. Furthermore, salts with chaotropic anions (thiocyanate, iodide, perchlorate) will affect the performance of the kit. Solutions with these salts will cause a precipitate to form as soon as reagent A is added. Chaotropic cations (guanidine) do not have this effect. Guanidine thiocyanate will affect performance of the kit but guanidine HCl will not. Sodium iodide and sodium perchlorate will affect performance but sodium chloride will not.

Do nucleic acids co-precipitate with proteins?
Yes nucleic acids do precipitate to some extent with this kit. 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.

The protocol gives a choice between acetone, acetonitrile and 70% ethanol for the wash steps. Which one is best?
The choice of washing solution will depend on the application. An acetonitrile wash may help the recovery of low molecular weight proteins while 70% ethanol may be the best wash where salt removal is of utmost importance. Different washes can also be done sequentially. The best washing procedure for your application may have to be determined empirically.

ND Protein Precipitation Kit	
EC-888	Precipitates 50ml