national diagnostics

ProtoGel® Sample Prep Kit

The ProtoGel Sample Prep Kit is a convenient, universal method for concentrating and optimizing protein samples prior to SDS-PAGE. The ProtoGel Sample Prep Kit ensures reproducible electrophoretic mobility in SDS-PAGE and excellent visualization results.

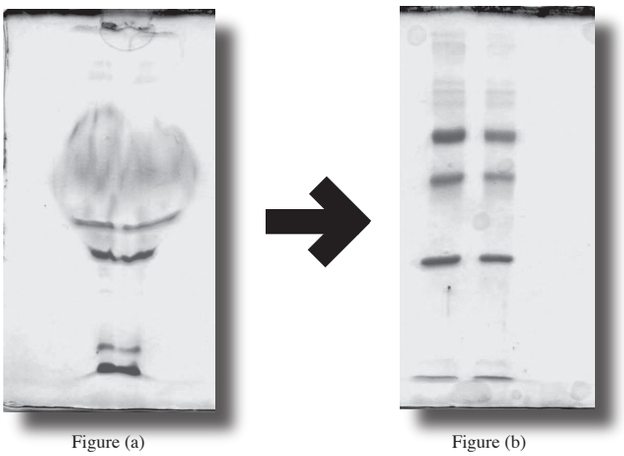


Figure (a) Figure (b)

Gels (a) before and (b) after the use of National Diagnostics' ProtoGel Sample Prep Kit.

Frequently Asked Questions

What is the lower concentration limit of protein that can be concentrated?
The lower limit for reproducible recovery of BSA is 100ng in 40μ1.

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

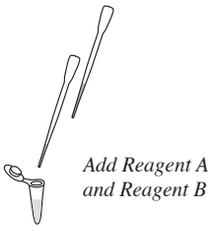
Does the concentration of salt in the sample have an effect on the results?
Most salts at concentrations used in biological laboratories will not affect the precipitation method. However, the surrounding solution can effect kit performance in a few instances. Very high salt concentrations e.g. a saturated solution of NaCl (5.5M) will make it difficult to collect the pellet due to the high density of the solution. In this case it may be helpful to dilute the sample before starting the precipitation.

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.

Are there any reagents that are incompatible with the ProtoGel Sample Prep Kit?
Deoxycholate or salts of thiocyanate or perchlorate are incompatible with the kit. SDS or nonionic surfactants such as Tween™ or Triton™ above 1% will reduce protein recovery substantially.

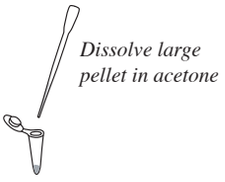
Protocol

1. Add 5 µL of Reagent A for every 100 µL of sample in a microcentrifuge tube and mix well.



2. Add 10 µL of Reagent B for every 100 µL of sample and mix.
3. Incubate for 20 minutes at room temperature, inverting the tube occasionally to promote mixing.

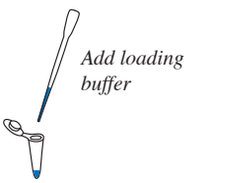
4. Collect complex by centrifugation at 12,000 x g and remove supernatant. The large white pellet contains the precipitant complex and the protein.



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.

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 µg.

7. Wash protein pellet at least twice by suspending the pellet in 70% ethanol and collecting proteins by centrifugation. **NOTE:** These washes are critical to the purity of the recovered protein.
8. Air-dry pellet, mix with Protein Loading Buffer Blue 2X and deionized water to desired volume, heat to 95 °C for two minutes and load onto SDS-PAGE gel.



ProtoGel Sample Prep Kit

EC-884 prepares 100 samples

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