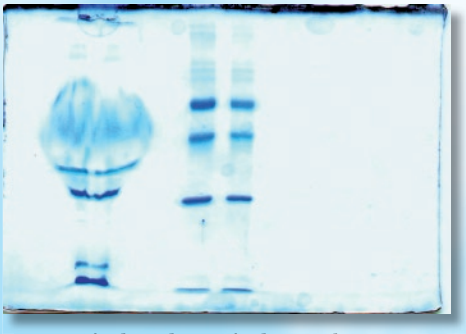
# ProtoGel® Sample Prep Kit

Patented system for routine sample preparation
for both pre-cast and traditional SDS-PAGE

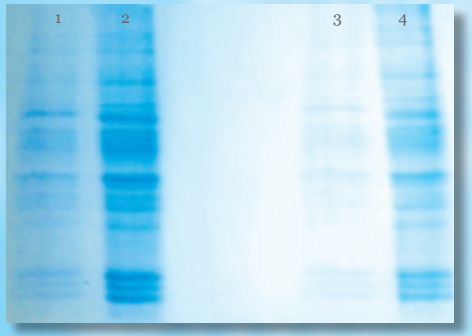
- Removes interfering contaminants
- Concentrates dilute samples
- Prevents gel failures
- Simple and inexpensive



*Unpurified and purified samples in SDS-PAGE.*

## Purification

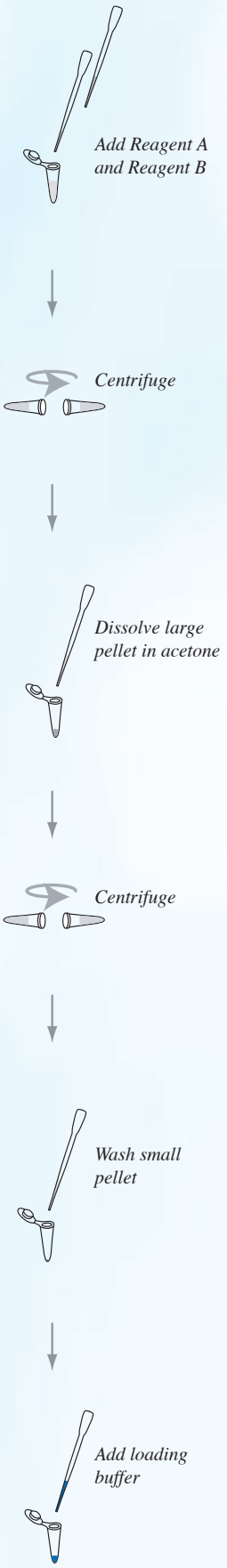
Contaminants in the sample such as high salt or urea lead to blurred bands or smiling gels in SDS-PAGE. With the ProtoGel Sample Prep Kit, interfering substances from upstream applications can no longer gain entry to the well. Contaminants are washed away with a simple method. The sample loaded contains only pure protein and loading buffer with no contaminants remaining to impede reproducible, high-resolution results.




*Lanes 1 and 3 are unconcentrated. Lanes 2 and 4 are concentrated.*

## Concentration

In addition to purification, proteins previously too dilute for SDS-PAGE can now be concentrated prior to electrophoresis with a simple method. The ProtoGel Sample Prep Kit concentrates proteins as dilute as 25ng/100µl. The patented ProtoGel Sample Prep Kit casts the finest net of any recovery system, concentrating all proteins in high yield regardless of identity. With the ProtoGel Sample Prep Kit, the purity and concentration of your SDS-PAGE samples are both under your control.




national diagnostics

**ProtoGel Sample Prep Kit** EC-884
1 Kit Purifies 200 100µl Samples

# Method of Use

The ProtoGel Sample Prep Kit represents a convenient, universal method for purifying and concentrating protein samples prior to SDS-PAGE.

The kit consists of National Diagnostics' patented surfactant system of Reagent A and Reagent B, as well as several vials of traditional Laemmli loading buffer. You may use an alternative loading buffer if recommended for your particular type of gel.

## 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 at a concentration of 100ng/400$\mu$1.

**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.

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. Thus, guanidine thiocyanate will affect performance of the kit but guanidine HCl will not, and similarly, sodium iodide and sodium perchlorate will affect performance but sodium chloride will not.

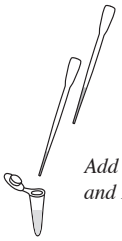
**Does the pH of the starting solution affect precipitation?**

The kit has been tested on protein solutions between pH 6 and pH 8 and no difference was seen in the recovery.

**How do I select the final wash: Acetone, Acetonitrile, or 70% Ethanol?**

For most samples, 70% Ethanol is ideal as it removes the most contaminants. Acetonitrile may be used in the unusual case where smaller proteins prove soluble in ethanol. Acetone is only recommended for extremely small proteins, as it will not completely elute contaminants.

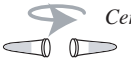
1. Add 1/20 volume Reagent A to sample in a centrifuge tube and mix well.



*Add Reagent A and Reagent B*

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.*



*Centrifuge*

4. Collect precipitate by centrifugation and remove supernatant. *The pellet will be large.*

*Dissolve large pellet in acetone*

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.*

*Centrifuge*

6. Collect proteins by centrifugation.

7. To remove salts and surfactants, wash pellet briefly with 70% Ethanol (or Acetone or Acetonitrile). *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.*

*Wash small pellet*

*Add loading buffer*

8. Combine pellet with Protein Loading Buffer Blue 2X and deionized water and load onto SDS-PAGE gel.