TCA Precipitation of Proteins

AAH with modifications from AJA and FS 6/10/96

This has two uses. One to concentrate the protein before running a gel. Second to remove materials which would affect the gel running (eg SDS gels run very poorly in potassium)

Basic Procedure

1. To the solution add an equal volume of 20% TCA.
2. Leave on ice 20mins.

   For high protein concentrations the protein will precipitate immediately. For low concentrations, leave longer.
3. Spin 15 mins in the microfuge with the lid hinge facing out so that you remember where the pellet is
4. Suck off all the sup with a drawn out pasteur pipette. It is important to remove all the TCA because otherwise it will acidify the sample buffer, making it go green
5. Optional step. You can wash in acetone at this stage to remove all the TCA. This can have the disadvantage of making a really dry pellet which is hard to resuspend. If the pellet is big it is recommended to do this step.
6. Resuspend in 2X sample buffer. If you have problems with resuspending the pellet, sonnicate with the micro tip sonnicator on the 5th floor, very gently.
7. If the sample goes green because of acidification, you can neutralize it by sucking up some ammonia vapour with a pasteur pipette and blowing it into the sample.

Modifications

Detergent in the sample

If the sample has detergents you want to avoid precipitating the detergent. In the first step, add 1 volume of 20% TCA, 80% acetone. The detergent will stay in the acetone phase

Very low protein concentration

- Add NP40 to 1%.
- Add 1 volume 20% TCA.

Continue as step 2, but including the acetone washing step.