

Stock solutions:

1. Sample buffer at pH 8 – 8.25 - Tris, HEPES or ammonium bicarbonate are appropriate buffers for this, among others, , without reductant (no DTT or BME). Can be the same as your “sample loading buffer” without BME or DTT, but it needs to be at PH 8 –8.5.⁴
2. 100mM DTT. 15.4 mg/ ml. Store frozen aliquots at -20C.
3. 300mM iodoacetamide in water. (Sigma Cat.# I1149, fw: 186g/mol). Dissolve 55.8 mgs iodoacetamide per 1.0 ml water. Prepare fresh, prior to use.

Reduction and alkylation:

1. Set a heating block to 55C.
2. Re-suspend protein pellet/collect sample in sample buffer without reductant and at pH 8 – 8.25 (see “Stock Solutions”). Vortex to mix.
3. Add DTT stock solution as 20x (5mM final), or 5.25ul of stock per 100ul of sample buffer. Vortex to mix.
4. Incubate for 30 minutes at 55C.
5. Remove from the heating block and allow to cool completely to room temperature. Spin the sample to return condensation to the bulk liquid in the bottom of the tube, and vortex occasionally to mix and redistribute heat. It is important that the sample be completely cooled prior to alkylation.
6. Add iodoacetamide solution as 20x (15mM final), or 5.25ul of stock per 100ul sample buffer. Vortex to mix.
7. Remove the sample to the dark for 1 hour at room temperature. A bench drawer works fine for this.
8. Add an additional aliquot of DTT stock solution as 20x. Vortex to mix. Allow to stand at room temperature for an additional 15 minutes.
9. The samples are ready for SDS-PAGE.

Notes:

1. Reduction using BME is not advised. Reduction using phosphines works fine.
2. Always use gloves when handling active iodoacetamide solutions, as they are toxic.
3. Be sure to allow the sample to come to room temperature after reduction – incubating them at elevated temperature with alkylating solution can result in alkylated lysine residues. In this regard, using a nucleophilic base buffer system (e.g. ammonium bicarbonate) assists in preventing inadvertent lysine alkylation.
4. It seems everyone who reads this protocol thinks “sample buffer”=what I call “loading buffer”, but it is not necessarily so. It is just something convenient to bring your sample up in as long as it is at pH 8-8.5 BUT you can do the reduction and alkylation in your loading buffer as long as you take into consideration the dilution factor of the added red/alkyl buffers. I do the reduction and alkyl. in 50 mM Ammonium bicarbonate. But I've got the luxury of working with pure proteins that I weigh out and pretty well know what the final concentrations are going to be. Those of you who are kind of winging it as far as composition/concentration of unknowns may have other buffering/solvating requirements, but if it were me I'd use as simplified a buffer as you can for red/alkyl and add the loading buffer in the correct proportions when you are ready to run the gel (in the end it will be the gel that removes any MS interfering components).