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Cysteine Alkylation Before SDS-PAGE

(The method is based on the publication by Salvatore Sechi and Brian T. Chait, Rockefeller University, with some modifications.)

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1. Add NuPAGE LDS Sample Buffer 4X (Invitrogen Cat. No. NP0007) to it make it 1X in concentration.

2. Add DTT to final concentration of 5mM. Incubate for 30 minutes at 65C.

3. Add iodoacetamide (Sigma Cat. No.16125-10G) to final concentration of 10 mM. Put the sample tube in an enclosed box (keep it in dark) and incubate at room temperature for 1 hour.

4. Load sample for SDS-PAGE.

Note:

1. Other sample buffer should also be working, although we have only tested the Invitrogen LDS Sample buffer. Please make sure the pH of a sample buffer at pH8.4. Some sample buffers have a pH around 6, which is not optimal for Cys reduction by DTT. You may also use recipe in Ref. to make your own sample buffer: 0.9 M Tris-HCl, pH 8.45, 24% glycerol, 8% SDS, 0.01% coomassie G, 0.01%phenol red. We suggest adding DTT separately since DTT in a sample buffer is not stable for long-term storage.

2. We suggest to make DTT 50mM stock solution, iodoacetamide 100mM stock solution, both in water, aliquot to 100 ul each in 0.6ml microtubes, and store at -40 freezer. They should be stable for a long time without multiple freezes and thaw.

3. Since some of iodoacetamide will be titrated by remaining DTT, iodoacetamide is added to 2X of DTT. The reaction is carried out in dark to avoid possible modification of proteins by some by-product of the reaction.

4. Please make sure the original pH of a protein sample is in a pH6-7 range, otherwise the sample buffer may not be able to raise it pH to 8 and DTT reduction reaction may not be complete.

Reference: Salvatore Sechi and Brian T. Chait, Anal. Chem. 1998, 70, 5150-5158, Modification of Cysteine Residues by Alkylation. A Tool in Peptide Mapping and Protein Identification.

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