Methylation Protocol Walter TS, Meier C, Assenberg R, Au K, Ren J, Verma A, Nettleship JE, Owens RJ, Stuart DI, Grimes J Lysine methylation as routine rescue strategy for crystallization Structure WAYS & MEANS 2006 Nov;14(11):1617-22.

Chemicals:

1. Formaldehyde solution
2. ABC (Borane dimethylamine complex) reducing agent

1. Exchange the protein solution into a Hepes buffer. example: 50mM Hepes pH7.5, 200mM NaCl. Tris, reducing agents (DTT, TCEP, mercaptoethanol) interfere with the reaction.

2. Concentrate the protein to 1mg/ml. Use fresh protein. The methylation is less efficient for old material.

3. Prepare stock solutions: 1M ABC: 60mg in 1ml H2O. Difficult to dissolve. Mix by gently pipetting up and down. Keep on ice. 1M Formaldehyde in water. Prepare 1ml. Keep on ice.

4. Methylation reaction: For 1ml protein at 0.5-1.0 mg/ml, add 20 ul of 1M ABC, add 40ul of 1M formaldehyde solution. Mix by gently pipetting up and down. Incubate on ice. Protein may precipitate at this step. In this case, repeat the methylation reaction with 0.5mg/ml or less.

After 2 hours, add 20 ul of 1M ABC, add 40ul of 1M formaldehyde solution. Mix by gently pipetting up and down. Incubate on ice. After 4 hours, add 10 ul of 1M ABC. Mix by gently pipetting up and down. Incubate at 4°C for 20H.

5. Stop the reaction. Exchange the buffer into a tris buffer to neutralize any methylation capacity left in the solution. Example 20mM Tris-HCl ph7.5, 200mM NaCl.

6. Gel-filtration Recommended step. Methylated protein tends to run differently on a GF.