# JBS Methylation Kit

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-510</td>
<td>6 reactions</td>
</tr>
</tbody>
</table>

For *in vitro* use only.  
Quality guaranteed for 12 months.  
Store at 4°C.

## Application

Reductive methylation of lysine residues to alter crystallization properties of proteins.

## Kit Contents

Each JBS Methylation Kit contains reagents sufficient for six methylation experiments:

1. **Stock A**: 6 solid aliquots of 6 mg Dimethylamine Borane Complex
2. **Solution B**: 1 ml of 1 M Formaldehyde
3. **Solution C**: 1 ml of 1 M Tris, pH 7.5
4. **Stock D**: 6 aliquots of 7.7 mg DTT

## Background

Growing well ordered single crystals is an essential step in order to determine the three-dimensional structure of a biological macromolecule by X-ray diffraction. Although, the crystallization screens of our JBScreen family have been successfully employed in many cases, there are proteins which are reluctant to crystallize.

Reductive methylation of free amino groups is a straightforward and very efficient method for improving/obtaining crystals. It has emerged as a standard procedure in several large scale facilities and research programs, i.e. the Midwest Center for Structural Genomics [1] and the Structural Proteomics In Europe (SPINE) program [2,3]. The difference observed in the crystal structure between the native and the methylated proteins are in the same range as the deviation between the structures of two different crystal forms of the same protein. Generally, methylated proteins retain their biochemical function [4,5].

Different protocols for the reductive alkylation of lysine residues in proteins have been reviewed [5,7]. Reductive methylation employing formaldehyde as alkylation reagent and dimethylamine borane complex as reducing agent has been shown to have the mildest effect on the biochemical properties of the protein [6].

The first step of the reaction involves the initial formation of a Schiff base between the ε-amino group of a lysine residue and formaldehyde which is subsequently reduced to a secondary amine Eq.(1).

Secondly, monomethyl lysine reacts rapidly with formaldehyde since the pKₐ of monomethyl lysine is slightly smaller than that of lysine itself Eq.(2).

Please be aware that the N-terminal residue of the protein backbone will be methylated as well.

![Chemical Reaction Diagram](image)

### Instructions

The instructions correspond to the reaction of 1 ml protein solution. The concentration of the protein should be in the range between 1- 10 mg/ml.

All reactions should be performed at 4°C. Prepare the reagents immediately before use and keep them on ice.

1. Dialyse your protein against a buffer at pH 7.5 containing no free amino groups and/or alcohols. If necessary, salts can be added to stabilize the protein since they do not interfere with the reaction. We recommend 50 mM HEPES or phosphate buffer (pH 7.5) at protein concentrations between 1-10 mg/ml.

2. Set up solution A by adding 100 µl of reagent grade water to one vial of stock A and mix gently at 4°C.

---

1 The protocol ensures an adequate excess of reagents even for proteins with a very high number of lysine residues at a concentration of 10 mg/ml. However, if the protein precipitates during the experiment, the concentration should be lowered.
(3) Add 20 µl of solution A to 1 ml protein solution.

(4) Immediately add 40 µl of solution B with gentle mixing. Incubate the reaction mixture at 4°C for two hours.

(5) Repeat steps (3) and (4) and incubate the reaction mixture for additional two hours at 4°C.

(6) Add a final aliquot of 10 µl solution A and leave the reaction mixture overnight at 4°C.

Please Note: In a few cases precipitation of the protein has been reported. Remove the precipitant by centrifugation before carrying on with the protocol.

(7) Add 125 µl of solution C to stop the reaction.

(8) Separate the protein from the reaction mixture by extensive dialysis, size exclusion chromatography, microfiltration, or precipitation.

(9) Prepare a 50 mM DTT stock solution by adding 1 ml reagent grade water to one vial of stock D. This stock solution is stable for several hours at 4°C.

(10) Add DTT stock solution up to a total final concentration of 1-5 mM to stabilize your protein.

Now you are ready to set up your crystallization experiments. Please note: Methylation can alter the surface of the protein in a way that the crystallization conditions for the native protein may not yield crystals of the methylated form [5].

The reductive alkylation of lysine residues can be monitored by mass spectroscopy.

References

Selected Literature Citations of JBS Methylation Kit
• Cima et al. (2012) Insight on an Arginine Synthesis Metabolon from the Tetrameric Structure of Yeast Acetylglutamate Kinase. PLOS one 7:e34734.

Fig. 1. MS Spectra of hen egg white lysozyme
a) native lysozyme and b) methylated lysozyme