**Using Global-Methylation Inhibitors**

The activity of AdoMet dependent methyltransferases can be inhibited by three types of small molecules: (1) analogs of AdoMet that compete for the cofactor binding sites, (2) compounds that target the metabolic loop of the universal methyl-donor AdoMet, and (3) specific methyltransferase inhibitors. Sinefungin is a commonly used AdoMet analogue, however it is not taken-up very efficiently by cells. Adenosine dialdehyde (AdOx) and 5'-methylthioadenosine (MTA) are often used as global methyltransferase inhibitors, which are cell permeable. AdOx is an AdoHcy hydrolase inhibitor that causes the accumulation of intracellular AdoHcy levels. This increase in AdoHcy levels results in feedback inhibition of most methylation reactions. MTA, a nucleoside inhibitor of methyltransferases, blocks the transfer of methyl groups from AdoMet to PRMT substrates. Recently, a large number of efforts are underway, both in academia and in pharma, to identify small molecule inhibitors that selectively inhibit specific PRMTs (Cheng et al., 2004; Purandare et al., 2008; Spannhoff et al., 2007). Although a number of specific PRMT inhibitors have been identified using *in vitro* screens, these compounds have proven to be of limited use due to their inability to enter cells or their cytotoxic effects. Generally, cells are cultured in the presence of AdOx to generate hypomethylated protein extracts that are good all-purpose *in vitro* substrates for methyltransferases (Lin et al., 1996). Here we demonstrate using AdOx in cell-based methylation assays. This approach is used to confirm that tritium labeling is indeed due to the transfer of a methyl group by an AdoMet-dependent methyltransferase (**Figure 1**).

1. A stock solution of AdOx (20 mM) is prepared in dimethyl sulfoxide (DMSO).
2. HeLa cells are grown on a 10 cm culture plate until they are 60% confluent.
3. The cells are then incubated with AdOx at a final concentration of 20 μM.
4. After 24 h of AdOx treatment, the cells are subjected to cell-based methylation labeling. The procedure is the same as described in **Cell-Based Methylation Assays**, except growth medium A and B are now supplemented with 20 μM AdOx.

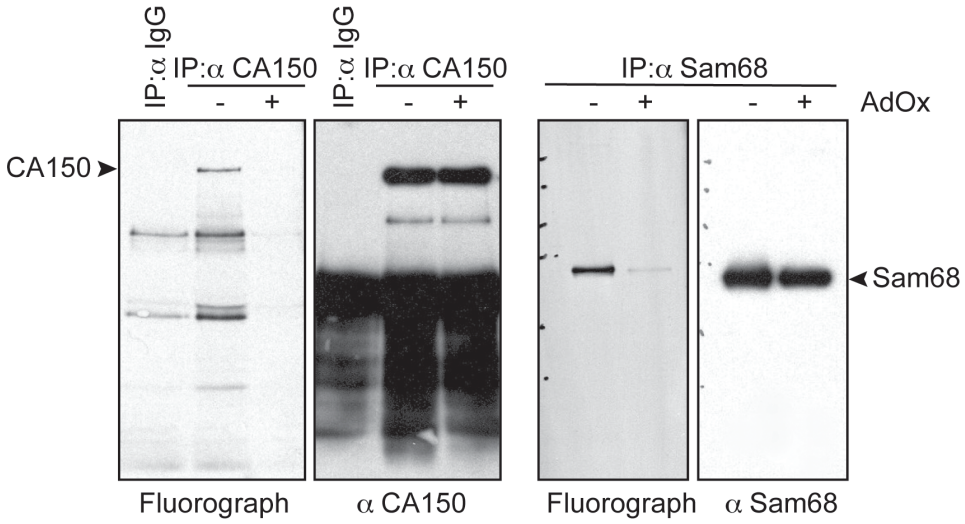


**Figure 1.** The methyltransferase inhibitor, AdOx, prevents CA150 and Sam68 methylation in cells. HeLa cells were grown for 24 h in the presence of 20 μM AdOx. These cells were then subjected to an *in vivo* methylation assay by tritium labeling, in the presence of 20 μM AdOx. IPs were performed with αCA150 or αSam68 antibodies. The $^{3}$H-labeled proteins were visualized by fluorography. After fluorography, the same membrane was washed in TBST and immunoblotted with αCA150 or αSam68 antibodies. We clearly see equal amounts of the proteins in cells that are treated and untreated with AdOx. But methylated bands are only seen in the untreated samples.

**References**

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