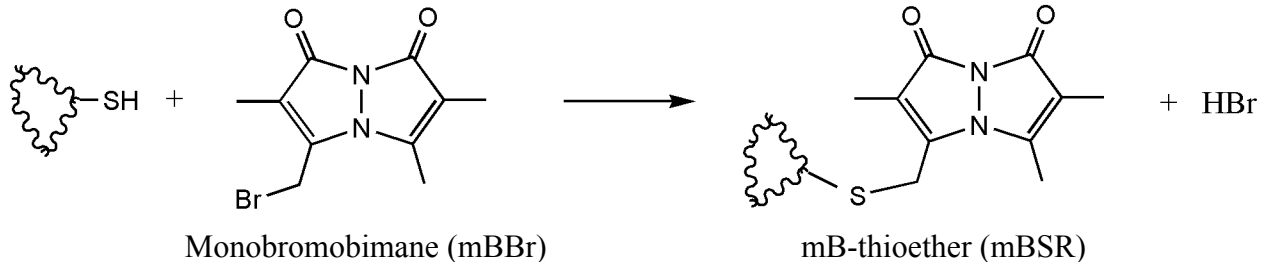


Thiol labeling with Monobromobimane (mBBr, 3,7-Dimethyl-4-bromomethyl-6-methyl-1,5-diazabicyclo-[3.3.0]-octa-3,6-dien-2,8-dion)



Primarily mBBr is used, because it reacts selectively with thiol groups yielding highly fluorescent and stable thioethers, which can be easily detected at the pmol level (R. C. Fahey, G. L. Newton, *Methods Enzymol.* 143, 85 (1987)). A helpful secondary effect is that mBBr labeling takes care of the otherwise often unusual migration behaviour of some proteins with a high cysteine content on SDS-PAGE and even facilitates Coomassie or silver staining of the gel (G. Meloni, pers. commun.).

Required stock solutions:

- 100mM mBBr in Acetonitrile (store in 20 μ L aliquots in the dark at -20°C)
- 1M Tris-HCl (pH 7.5)

■ prepare the following sample

	sample
Solution containing SH groups	20 μL
100mM mBBr	0.44 μL^*
1M Tris-HCl (pH 7.5)	1 μL
ddH ₂ O	0.56 μL
	22 μL

* if you have more than 2mM SH groups in your solution adjust accordingly!

- let react in the dark at room temperature for 30-60 minutes
- remove unreacted mBBr by extraction with dichloromethane (CH_2Cl_2)
add 100 μL CH_2Cl_2 , vortex, remove CH_2Cl_2 (lower phase), repeat 2-3 times
- for SDS-PAGE add 22 μL [2xTSB buffer and proceed as usual](#)