



Protocols for Blocking Endogenous Peroxidase Activity

- Method 1** 3% H₂O₂ in water. Incubate for 5 minutes. Rinse with water 2-3 minutes.
This is the most rapid and simple technique for quenching, however the bubbling that might occur may damage the morphology of frozen sections and specimens with large amounts of endogenous enzyme activity (eg., blood smears, etc.). This is a good general block.
- Method 2** 0.3% H₂O₂ in methanol. Incubate for 20-30 minutes. Rinse with water 2-3 minutes.
This is the method of choice for frozen sections and specimens with large amounts of endogenous enzyme activity (blood smears, cytopspins, etc.). The concentration of H₂O₂ can be doubled and/or incubation time shortened as appropriate for the specimen. Methanol accelerates the destruction of the heme groups so a lower concentration of H₂O₂ can be used for a longer period of time. This is also a good general block except for cell surface markers.
- Method 3** 0.180 g β-D(+) glucose, 0.005 g glucose oxidase, 0.0065 g sodium azide in 50 ml PBS. Incubate sections for 1 hour at 37 °C. Rinse in PBS 3 x 5 minutes.
*This reaction slowly and steadily produces very low concentrations of H₂O₂ by enzymatic reaction and has been found to be preferable to preformed H₂O₂ because inhibition of peroxidase activity was found to be consistently complete. ref: Andrew S.M., Jasani, B. (1987) *Histochem J.* **19**: 426-430.*
- Method 4** 0.3% H₂O₂ in 40% methanol (in PBS) overnight.
This is a good procedure for preserving some membrane markers in hematopoietic tissues.
- Method 5** 100% ethanol fixation followed by .075% HCl (0.2 ml concentrated HCl in 100 ml ethanol). Incubate for 15 minutes at room temperature.
- Method 6** 0.01% periodic acid for 10 minutes followed by sodium borohydride treatment (0.1 mg/ml water) for 2 minutes to reduce aldehydes generated.
- Method 7** 0.05% sodium azide mixed into the DAB/ H₂O₂ solution.
- Method 8** 0.1% phenylhydrazine for 60 minutes at 37 °C
- Method 9** 1% sodium nitroferricyanide in absolute methanol containing acetic acid.