VECTOR LABORATORIES

Protocols for Blocking Endogenous Peroxidase Activity

Method 1 BLOXALL™ Blocking Solution (SP-6000) is provided ready-to-use in a dropper bottle. Prior to the application of primary antibody, apply BLOXALL™ Blocking Solution to the section. Incubate for 10 minutes at room temperature. Wash sections in buffer for 5 minutes.

BLOXALL™ Blocking Solution inactivates endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations.

Method 2 3% H_2O_2 in water. Incubate for 5 minutes. Rinse with water 2-3 minutes.

This is a rapid and simple technique for quenching, however bubbling that might occur may damage morphology of frozen sections and specimens with large amounts of endogenous enzyme activity (eg., blood smears, etc.). This is a good general block.

Method 3 0.3% H_2O_2 in methanol. Incubate for 20-30 minutes. Rinse with water 2-3 minutes.

This method is often used for frozen sections and specimens with large amounts of endogenous enzyme activity (blood smears, cytospins, etc.). The concentration of H_2O_2 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 H_2O_2 can be used for a longer period of time. This is also a good general block except for cell surface markers.

Method 4 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 H_2O_2 by enzymatic reaction and has been found to be preferable to preformed H_2O_2 because inhibition of peroxidase activity was found to be consistently complete. ref: Andrew S.M., Jasani, B. (1987) Histochem J. 19: 426-430.

Method 5 0.3% H_2O_2 in 40% methanol (in PBS) overnight.

This is a good procedure for preserving some membrane markers in hematopoietic tissues.

Method 6 100% ethanol fixation followed by .075% HCl (0.2 ml concentrated HCl in 100 ml ethanol). Incubate for 15 minutes at room temperature.

Method 7 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 8 0.05% sodium azide mixed into the DAB/H_2O_2 solution.

Method 9 0.1% phenylhydrazine for 60 minutes at 37 °C

Method 10 1% sodium nitroferricyanide in absolute methanol containing acetic acid.

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