Inhibition of endogenous enzyme activity

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Nonspecific staining in immunohistology is a multifactorial problem and may occur to variable degrees. A major problem of so-called “background” reactions can be encountered by the activity of endogenous enzymes. In certain cases, they will affect the interpretation of results. Appropriate controls are always necessary to judge interferences and to recognize the underlying reasons of nonspecificity. In the case of enzymes as markers, endogenous enzyme activities (e.g. peroxidases, alkaline phosphatases) must be expected. Usually, they should be blocked to enable clear-cut immunostaining. The selected techniques must be gentle enough not to destroy the molecules to be studied. To this end, the effects of quenching procedures have to be carefully controlled.

Peroxidase (HRP) from horseradish, glucose oxidase (GOD) from *Aspergillus niger* and alkaline phosphatase (AP) from calf intestine are the most employed enzymes for cellular labeling purposes. Endogenous peroxidases occur in many tissues, and their inhibition is usually necessary in immunoperoxidase experiments. Because glucose oxidase does not occur in mammalian species, inhibition treatmens are not needed in the respective preparations. Alkaline phosphatases are known to occur in a variety of tissues, schedules of inhibition are recommended.

We favor hydrogen peroxide in immunoperoxidase labelings to inhibit endogenous peroxidases, but other inhibitory solutions may work as well. Hydrogen peroxide can be used in varying concentrations and is readily prepared from a concentrated commercial stock solution (e.g. 30% H_2O_2) in distilled water or in buffer (usually phosphate buffered saline). The concentrations needed for inhibition will vary and depend on the material under study. This quenching step is usually performed prior to the application of primary antibody, but this step can be also done after incubation in link antibodies.

Due to the lack of endogenous enzyme activities, glucose oxidase is a useful marker enzyme for mammalian tissues; comparable inhibition procedures as with peroxidases are not needed. This can be of advantage when very high concentrations of endogenous peroxidases, such as in eosinophils, are present, and when they are difficult to quench, or when deleterious effects by inhibitors are feared.

With the introduction of levamisole, the inhibition of the nonintestinal forms of alkaline phosphatse are no longer a problem. This inhibitor is added to the final substrate mixture while the AP isoenzyme from calf intestine (as marker enzyme) is not affected.

For enzyme inhibition formulations and other blocking schedules, see *Blocking solutions* in chapter *Reagents*.

Selected publications for further readings

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Full citation of publications is given in chapter *References*
link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf

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