

Gold labeled reagents as marker system

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Apart from fluorochromes and enzymes, some other markers are available which can be used for immunohistology with main interest in ultrastructural studies. For electron-optical reasons, primary electron dense molecules or particulate substances with characteristic size and shape are candidates of first choice (see *Immunohistochemical marker molecules*, and *Markers for immuno-electron microscopy*). One of such markers is colloidal gold (FAULK WP and TAYLOR GM, 1971; ROTH J et al., 1978). Even if originally developed for the electron microscope, gold labels have also proved useful for the light microscope.

Colloidal gold particles bind noncovalently to proteins at pH values near the protein's pI. In the meantime, a wide range of proteins such as antibody molecules, protein A and streptavidin have been adsorbed with uniform gold particles of various sizes. Gold labels avoid the problems of enzyme cytochemical substrate preparation and endogenous enzyme activity. The lack of sensitivity at the level of light microscopy has been overcome by recent developments in signal amplification by photochemical silver method.

Gold labels can be detected in the light microscope by bright field illumination. Labels range from pale pink to deep red, depending on the strength of reaction. With modern silver enhancement methods, the gold particles become coated in metallic silver giving a brown-black color. The reactions can be readily controlled by direct and continuous monitoring in the microscope. Gold labels are compatible with many histological and histochemical stains. A range from ultra small colloidal gold reagents (<1 nm) to gold reagents in the order of >25 nm are now commercially available.

Also available are *gold*-mono(sulfosuccinimidylester) and *gold*-monomaleimide which can be conjugated to amines and thiols, respectively, in the same way as dyes are conjugated with antibodies or nucleic acids. Conjugates coupled to both a gold label and a fluorophore enable correlative studies with the fluorescence, light and electron microscopy. Gold conjugates have some advantages over colloidal gold inasmuch as they are extremely uniform ($1.4 \text{ nm} \pm 10\%$) and stable and they develop better with silver than most gold colloids, thus, provide higher sensitivity. Furthermore, gold conjugates have lower affinity for proteins than gold colloids, thereby reducing background in tissue sections due to nonspecific binding.

For optimal visualization of gold stainings in both light and electron microscopy, a convenient light-insensitive silver enhancement is recommended. Several silver enhancement kits are available. The advantages of silver enhancements include (a) high-contrast signal; (b) high sensitivity; (c) light-insensitive development procedure under normal room lighting; and (d) slow development of metallic silver coating for precise monitoring under the microscope.

Selected publications for further readings

Faulk WP and Taylor GM (1971)
Horisberger M and Rosset J (1977)
Roth J *et al.* (1978)
Danscher G (1981)
Holgate CS *et al.* (1983)

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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