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De-paraffinize tissue samples for staining or immunohistochemical procedures

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Introduction

Histochemical and immunohistochemical staining make it possible to visualize the distribution and localization of specific cellular or tissue components within a tissue sample. Histochemical staining involves applying chemical stains that react or bind to specific component structures within the cell or tissue, allowing these to be visualized using light microscopy. Examples of histological stains for detecting cellular compartments or tissue structures include: silver stains for elastic fibers; Hematoxylin for staining negatively-charged structures, such as nucleic acids in the nuclei of cells; and eosin, which binds to positively-charged molecules and is often used as a counterstain to Hematoxylin.

Immunohistochemical staining is achieved using a primary antibody that is specific for an antigen in the cell or tissue sample. To detect the primary antibody, it is labeled with biotin, a fluorophore or an enzyme. Alternatively, a labeled secondary antibody is used. If the antibody is biotinylated, an additional incubation step with a fluorophore- or enzyme-labeled avidin, streptavidin or NeutrAvidin Protein is required. When enzyme labels are used, a precipitating substrate is added to produce an intensely colored product for analysis by light microscopy. When a fluorescent label is used, direct detection is accomplished using fluorescence microscopy. (These same detection procedures also can be used to visualize specific antigens in individual cells, a technique known as immunocytochemistry.) When using precipitating substrates, researchers often combine techniques of both immunohistochemistry (to identify a particular antigen) and histochemistry (to localize the antigen relative to other tissue landmarks).

Several methods exist for processing tissue samples for histochemical or immunohistochemical staining, including fixation with crosslinking reagents such as formaldehyde or glutaraldehyde, or alcohol or organic solvent denaturants. After fixation, tissues are frozen or embedded in paraffin for long-term storage. Although the paraffin wax preserves the tissue and its internal structures, it must be removed before staining so that the water-soluble staining solutions can effectively penetrate the tissue. The following procedure is for removing the paraffin from tissue samples that have been sectioned on a microtome and mounted onto glass slides. Mounted tissue samples are stable in their dry, embedded form until the paraffin has been removed. After de-paraffinization, the tissue must remain wet until staining is complete, at which time an appropriate mounting medium can be applied to affix a coverslip for visualization using a light or fluorescence microscope.

Procedure for De-paraffinization of Embedded Tissues

1. Incubate slides for 5 minutes in clean xylene;* repeat once.
2. Incubate slides for 5 minutes in 100% ethanol; repeat once.
3. Incubate slides for 5 minutes in 95% ethanol: 5% water; repeat once.
4. Incubate slides for 5 minutes in 85% ethanol: 15% water.
5. Incubate slides for 5 minutes in 70% ethanol: 30% water.
6. Incubate slides for 5 minutes in 50% ethanol: 50% water.
7. Incubate slides for 5 minutes in 30% ethanol: 70% water.
8. Incubate slides for 5 minutes in ultrapure water; repeat once.

The slides are now ready for staining or immunohistochemistry. Do not allow slides to dry. Store slides in a wash buffer, such as PBS or TBS (see Related Products), until ready to use.

*To avoid using organic solvents, substitute Fisherbrand™ Citrisolv™ Clearing Agent (Fisher Product No. 22-143-975, 1 L; and 22-143-976, 5L).

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