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CYTONEL KIT TROUBLESHOOTING GUIDE (Cytonel-Plus and Cytonel-19)

Problem 1:
No staining reaction seen

Cause:
Incorrect execution of the staining protocol.

Remedy:
Make sure that you follow the staining protocol steps in correct order.
Performance of the kit can be verified with the control slides included in the demo kit (slides available also at request). Compare your staining results of the control slide with the images below.

Problem 2:
Too weak brown staining reaction.

Cause 1:
Slide too wet when adding DAB.

Remedy:
Dry (blot) slide properly, or, as recommended, use Slide Spinner.

Cause 2:
Temperature of the thermal plate too low.

Remedy:
Adjust thermal plate to +42C.

Problem 3:
Unwanted cells (macrophages) display strong cytoplasmic staining (see image)

Cause:
Insufficient blocking of endogenous peroxidase activity.

Remedy:
Make sure that you prepared the fixative working solution as instructed.
Add 1 ml of 3% H2O2 and 0.5 ml blocking agent (included in the kit) in 100ml of commercial grade acetone. Use fixative within 6 hours. Please note that 30% stock solution of H2O2 may precipitate in the fixative.

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Problem 4:
Brown background on empty glass area.

Cause:
Some slide coatings may absorb staining reagents.

Remedy:
Avoid poly-L-lysine coated objective slides (SuperFrost Plus recommended).

Problem 5:
Brownish background staining on tissue section.

Cause:
Insufficient washing between antibody and DAB steps.

Remedy:
Wash slides for 10 sec with manual agitation. Make sure that you use PBS-0.05% Tween20. Washing buffer concentrate (tablet included in the kit) should be dissolved in 1 liter of distilled water.

Problem 6:
Weak staining reaction in tissue sections stored at -20C for long periods (months, years).

Cause:
Freeze-drying.

Remedy:
For longer periods unstained frozen sections (slides) need to be stored airtight (and preferable at -70C).

Problem 7:
Cytoplasmic fibers of the dendritic reticulum cells stain positively in sentinel nodes.

Cause:
Expression of cytokeratin 8 in these cells. These cells are occasionally stained with Cytonel-Plus (pancytokeratin) kit. The phenomenon is well-documented in the histopathology literature.

Remedy:
Keep in mind during histopathologic examination. Carcinoma cells have typical round or oval morphology. Cells with fiber-like elongations should be ignored.

Remedy 2:
Use Cytonel-19, which reacts only with cytokeratin 19 and not with cytokeratin 8.