Methyl green counterstaining

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Subsequent to immunohistological procedures, counterstaining of tissue sections with methyl green is a useful alternative to haematoxylin, carmine and other dyes for nuclear staining.

Methyl green has seven methyl groups. The seventh methyl group is easily lost and the dye reverts to crystal violet. Thus, there is invariably a small quantity of crystal violet mixed with the methyl green. The commercial product *Methyl green* 'CERTISTAIN' for microscopical staining from the Merck group (Merck, BDH/Gurr and EM Diagnostic Systems) is a closely related dye, Ethyl green, in which the seventh methyl group is replaced by an ethyl group (cf. to the homepage of BD LLEWELLYN (http://www.stainsfile.info/StainsFile/bdl.htm).

There exist several formulations for methyl green staining. A useful procedure is described here.*

Methyl green

Chemicals	**Chemical solution**
Methyl green (C.I. 42585) or Ethyl green (C.I. 42590) a closely related dye Sodium acetate trihydrate Glacial acetic acid Ethanol Distilled water	• 0.1 M acetate buffer pH 4.2: 1.36 g sodium acetate dissolved in 50.0 mL distilled water adjust pH to 4.2 with 0.1 M acetic acid and add distilled water to give a final volume of 100.0 mL • Methyl green stock solution: 1.0 g methyl green dissolved in 100.0 mL distilled water (warm) wash with chloroform to extract crystal violet (impurity) and filter • Methyl green dye solution: 50.0 mL methyl green stock *plus* 50.0 mL sodium acetate buffer

Staining procedure

Immuno-stained sections are passed through distilled water and stained:

– Methyl green dye solution	5-10 min
– distilled water	several rinses under microscopic control

* Dyes and other chemicals in histological staining can be toxic. They must be handled with care

–	differentiate in 70% ethanol	under microscopic control (sections turn green ∗)
–	96% ethanol	short dip
–	absolute ethanol	1 min

Slides are cleared in xylene or xylene substitute and mounted in resinous medium under coverglass

∗ Ethanol used for dehydration will remove some of the stain

References for further readings

Pappenheim A (1899)
Pappenheim A (1908)
Pollister AW and Leuchtenberger C (1949)
Kurnick NB (1952)
Baker JR and Williams EGM (1965)
Romeis B (1968)

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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