METHYL GREEN PYRONIN
04 - 121812

IVD In-vitro diagnostic medical device
CND Code: W01030799

number of tests ...................... 100
procedure time ...................... 45 minutes
product validity ...................... 2 years
storage temperature .................. 15-25°C
complementary equipment .......... not requested

Expected aim
Product for the preparation of cyto-histological samples for optical microscopy.

Application
To demonstrate simultaneously DNA and RNA in histologic sections. Recommended to show plasma cells and RNA in histologic sections and cytologic preparations.

Principle
In this method staining is obtained with a mixture of 2 basic dyes: purified methyl green and pyronin Y. In order to obtain a differential stain, a buffer is added to the solution to reach pH 4.8. If pH is lower than 4.8 a red stain caused by pyronin prevails; if pH is higher a green-blue stain caused by methyl green prevails. The 2 dyes are not intrinsically affine to DNA and RNA; their selectivity is a consequence of a definite pH value. This procedure may be difficult; we advise to read warning notes carefully and to work in as good conditions as possible.

Method
1) Deparaffinize section and bring to 70° ethanol.
2) Put 10 drops of reagent A on the section: leave to act 10 minutes.
3) Drain the slide and put on the section 10 drops of reagent B: leave to act 15 minutes.
4) Drain the slide and put on the section 10 drops of reagent C: leave to act 3 minutes.
5) Wash the slide in running tap water 10 minutes.
6) Rinse in distilled water.
7) Put on the section 10 drops of reagent D: leave to act 7 minutes.
8) Rinse quickly in distilled water and dry the slide with filter paper first, then the air for 10 minutes.
9) Clear twice in xylene and mount.

Results
DNA ........................................................................................................................... ................Pale green
RNA ........................................................................................................................... ................Pink to red
Mastzellen granules ........................................................................................................... ..................Blue
Background ................................................................................................................................. Turquoise

Reagents
A) Acid alcoholic buffer ....................................................................................................... 30 ml
B) Alcian blue alcoholic solution ........................................................................................ 30 ml
C) Acid alcoholic differentiation buffer ............................................................................... 30 ml
D) Methyl green pyronin buffered solution ....................................................................... 30 ml

Warning and precaution
Do not use fixatives with a formaldehyde concentration higher than 10% since higher concentrations block DNA aminic groups. Never use very acid fixatives: they would arrest reaction and cause hydrolysis. It is essential to avoid to use high temperature during paraffin impregnation and especially during flotation bath. Otherwise the distance between phosphoric groups in DNA molecule becomes too great for methyl green to be able to act on them (pyroninophilia of DNA).

The product must be used exclusively by specialized technical operators.
The product is classified as hazardous.
Read with attention the information written on the label (dangerous symbols, risks and safety phrases). Consult always the safety data sheet where the information about the risks of the preparation, precautionary measures during use, first aid and disposal are available. Do not use if primary packaging is damaged.

Storage
Store the preparation at room temperature. Keep the containers tightly closed.

Stability
After the first opening, the product is usable until the expiry date, if correctly stored.

Disposal
Hazardous preparation: observe all state and local environmental regulations regarding waste disposal.

References

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