

Papanicolaou EA65 Stain Solution

Introduction

The Papanicolaou stain is the gold standard in cytology. Initially developed to assess estrogen levels in vaginal epithelium and to detect pathogens such as *Candida* and *Trichomonas* in the genital tract, this method uses a combination of Orange G6 and either EA36 or EA50. This staining protocol produces vivid green, blue, and pink cytoplasmic coloration. Key features include bright, transparent cytoplasm and sharp visualization of nuclear membranes, nucleoli, and chromatin structures. The Pap stain is particularly effective for exfoliated cell specimens, rendering nuclei blue to black while staining the cytoplasm of keratinizing squamous cells pink to orange-red.

As a variant of the Pap stain, the Papanicolaou EA65 Stain Solution is preferred over EA36 and EA50 for staining exfoliated cells from non-gynecological samples.

Storage

Store at room temperature, stable for 1 year.

Protocol

1. Fix cell smears in 95% ethanol for 10-15 min.
2. (Optional) Immerse in 75% ethanol for 1 min.
3. (Optional) Immerse or rinse in distilled water for 1 min.
4. (Optional) Stain with hematoxylin staining solution for 5-10 min. Rinse with distilled water for 2 min.
5. (Optional) Differentiate in 1% hydrochloric acid-ethanol solution for about 4-5 sec, or in 0.5% hydrochloric acid aqueous solution for 10 sec.
6. (Optional) Rinse with tap water for 2 min, blue in bluing solution for 2 min, then rinse again with tap water for 2 min.
7. (Optional) Cover the smear with 95% ethanol for 5-10 sec to equilibrate.
8. (Optional) Stain with Orange G6 staining solution for 2 min. Rinse with 95% ethanol for 5-10 sec to remove excess stain.
9. Stain with EA65 staining solution for 3-5 min. Rinse with 95% ethanol for 5-10 sec to remove excess stain.

10. Dehydrate in absolute ethanol twice, 1 min each.

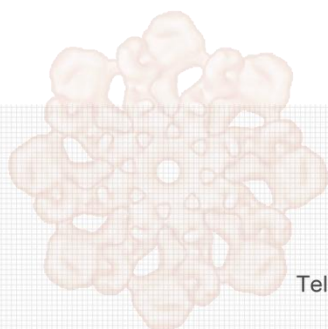
11. Clear in xylene and mount with neutral balsam.

12. Staining results

Nucleus	Blue-purple or black
Cytoplasm of non-keratinized cells	Light blue or light green
Cytoplasm of keratinized cells	Pink or orange-red

Note

1. Filter the staining solution before use. Replace frequently if reused.
2. This product contains a certain proportion of alcohol components. Cap tightly to prevent evaporation during use.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.



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