

## Papanicolaou EA36 Stain Kit

### Introduction

The Papanicolaou stain is the established standard in cytology. Originally developed to evaluate estrogen levels in vaginal epithelium and to detect pathogens such as *Candida* and *Trichomonas* in the genital tract, this method combines Orange G6 with either EA36 or EA50. This formulation produces vivid green, blue, and pink cytoplasmic staining. 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.

The Papanicolaou EA36 Stain Kit utilizes the EA36 solution for cytoplasmic staining and pairs it with a non-toxic, modified Hematoxylin solution for nuclear counterstaining. Compared to EA50, the EA36 solution is preferred specifically for gynecological cytology, making it ideal for cervical cancer screening and the detection of precancerous lesions.

### Storage

Components	Size	Storage
Reagent (A): Hematoxylin dye solution	100 mL	Room temperature protected from light
Reagent (B): Bluing solution	100 mL	Room temperature
Reagent (C): Orange G6 dye	100 mL	Room temperature protected from light
Reagent (D): EA36 staining solution	100 mL	Room temperature protected from light
Shipping: Blue ice	Shelf life: 12 months	

### Protocol

1. Fix cell smears in 95% ethanol for 10-15 min.
2. Immerse in 75% ethanol for 1 min. Immerse or rinse in distilled water for 1 min.
3. Stain with Hematoxylin dye solution for 5-10 min. Rinse with distilled water for 1 min.
4. (Optional) Differentiate in 1% hydrochloric acid-ethanol solution for about 4-5 sec, or in 0.5% hydrochloric acid aqueous solution for 10 sec.
5. Blue in bluing solution for 3-5 min. Rinse with distilled water for 1 min.

6. Cover the smear with 95% ethanol for 5-10 sec to equilibrate.
7. Stain with Orange G6 dye for 2 min. Rinse with 95% ethanol for 5-10 sec to remove excess stain.
8. Stain with EA36 staining solution for 3-5 min. Rinse with 95% ethanol for 5-10 sec to remove excess stain.
9. Dehydrate in absolute ethanol twice, 1 min each.
10. Clear in xylene and mount with neutral balsam.

**11. 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. If nuclear staining is not required, steps 3, 4, and 5 may be omitted, but the rehydration process cannot be skipped.
2. Do not allow the slide to dry out during steps 7 and 8, as this will easily cause precipitation and affect microscopic observation.
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.



**APExBIO Technology**  
[www.apexbt.com](http://www.apexbt.com)  
 7505 Fannin street, Suite 410, Houston, TX 77054.  
 Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com

