

## PAP (Papanicolaou) Stain- Harris Hematoxylin

### PRODUCT INFORMATION:

SSP005 100ml Ready to use  
 SSP005 250ml Ready to use  
 SSP005 500ml Ready to use  
 SSP005 1L Ready to use

### PERFORMANCE CHARACTERISTICS:

**Staining Interpretation:**  
**Nuclei** : Blue  
**Keratinized cells** : Orange  
**Superficial cells** : Pink  
**Erythrocytes** : Dark pink  
**Parabasal cells** : Greenish blue  
**Intermediate cells** : Greenish blue  
**Metaplastic cells** : May be greenish blue and pink

### SUMMARY AND EXPLANATION

#### For laboratory use only

The PAP (Papanicolaou) Stain kit is designed to differentiate between a variety of cells in vaginal smears for detection of vaginal, uterine and cervical cancer. In addition, this procedure is valuable for staining a variety of other bodily secretions and cell smears. The procedure was developed in the early 1940's by George Papanicolaou, the father of Cytopathology

### PRINCIPLE OF THE PROCEDURE

This technique uses a number of dyes in three solutions.

- **Hematoxylin:** The basic dye Hematoxylin is the nuclear stain which stains cell nuclei blue. It has the affinity for the negatively charged sulphate groups of chromatin on the DNA in the nuclei.
- **Orange G6:** is the first acid counter stain containing two sulphonic groups due to phosphotungstic acid-orange G compound. This bind to basic protein, such as prekeratin present in the cytoplasm of keratinized cells. Thus, the cytoplasm of keratinized cells stains orange color in different intensities.
- **EA50:** is second acid counter stain with two dyes-Eosin Y and Light Green SF. Eosin Y is fluorescent acidic dye binds to basic compound like proteins, and stains them dark red to pink as a result of action of bromine on fluorescein. It also stains collagen, muscle fibers, and erythrocytes to pink. Light Green SF is an atmospheric triarylmethane dye with C<sub>2</sub>H<sub>5</sub>N<sup>+</sup> reactive group, possesses an affinity for ribonucleic acid of ribosomes, which are abundantly present in prekeratinized cell. It stains prekeratinized or non-keratinized squamous cells, columnar cells into greenish blue.

### REAGENTS PROVIDED

Kit Contents	Product Code	Storage Conditions	Pack Sizes			
			100ml	250ml	500ml	1L
Harris Hematoxylin (Reagent A)	PS021	RT	100ml	250ml	500ml	1L
Orange G6 (Reagent B)	SS001	RT	100ml	250ml	500ml	1L
EA50 (Reagent C)	SS002	RT	100ml	250ml	500ml	1L

### STORAGE AND HANDLING

**Storage Recommendations:** Store at Room Temperature. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after expiration date provided on the vial.**

To ensure proper reagent delivery and stability, replace the dispenser cap after every use and immediately place the vials at room temperature away from sunlight in an upright position.

### SPECIMEN PREPARATION

**Recommended positive controls:** Gynecological smears or any superficial cell smears

DS-SSP005-B

### Sample preparation and fixation:

- A thin layer of cells smeared on the microscopic glass slides
- Fix the cell smears in 95% alcohol for 20 minutes

### PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed
2. This product should be used by qualified and trained professional users only
3. The product contains Alcohol and is classified as highly-flammable, must be kept away from ignition sources
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
5. Dispose of waste observing all local, state, provincial or national regulations.
6. Do not use reagents after expiration date
7. Use protective clothing and gloves, while handling reagents
8. Avoid microbial contamination of reagents as it may lead to incorrect results

### MATERIALS REQUIRED,BUT NOT PROVIDED

- Xylenes
- Graded alcohols (50%, 70%, 95%, Absolute)
- Bluing solution
- DPX Mountant
- Microscopic slides (Positively charged)
- Slide holder
- 1% Acid Alcohol
- Cover slips
- Coplin jars

### STAINING PROCEDURE

1. Place the smears in 95% alcohol for 20 minutes as part of fixation.
2. Rehydrate the slides in graded alcohols - 80%, 70%, 50% and distilled water for 2 minutes each.
3. Apply adequate amount of Harris Hematoxylin (Reagent A) to completely cover the smear for 5 minutes.
4. Rinse the slide in tap water for 2 minutes.
5. Dip in 1% Acid alcohol (optional) and wash in running tap water.
6. Rinse slide in distilled water for 2 changes.
7. Place the slide in 95% alcohol for 2 changes and 2 minutes each.
8. Cover the smear with adequate amount of Orange G6 (Reagent B) solution for 3 minutes.
9. Rinse the slide in two changes of 95% alcohol for 30 seconds each.
10. Stain the slide with EA50 (Reagent C) for 10 minutes.
11. Rinse the slide in 95% alcohol for 2 changes and 2 minutes each.
12. Quickly dehydrate the slide in 3 changes of absolute alcohol for 30 seconds.
13. Clear the slide in 2-3 changes of xylenes, 20 dips in each.
14. Cover slip with compatible mounting medium.

### QUALITY CONTROL

The recommended positive tissue control for PAP stain is gynecological or any superficial cell smears.

### PERFORMANCE CHARACTERISTICS

PAP Stain for Nuclei stains in Blue color, Keratinized cells stains in Orange color, Superficial cells stains in Pink color, Erythrocytes stains in Dark pink, Para basal cells stains in Greenish blue, Intermediate cells stains in Greenish blue and Metaplastic cells may stain in greenish blue and pink color.

### TROUBLESHOOTING

1. Follow the specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results
3. Do not allow the section to dry out during the entire staining process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results

5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: [techsupport@pathnsitu.com](mailto:techsupport@pathnsitu.com)

#### LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

#### BIBLIOGRAPHY

1. Papanicolaou, G.N. Atlas of Exfoliative Cytology, Harvard University Press, Cambridge, 1954.
2. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6<sup>th</sup> ed. Oxford: Churchill Livingstone Elsevier, 2008. 127-128.
3. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3<sup>rd</sup> edition. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 361-363.

#### EXPLANATION OF SYMBOLS

 LOT- Lot number / Batch number



Expiry

RT- Room Temperature

*Laboratory Use Only*