

Papanicolaou (PAP-NA) Stain Kit

PRODUCT INFORMATION: **PERFORMANCE CHARACTERISTICS:**

REF

SSP016-NA 100ml
 SSP016-NA 250ml
 SSP016-NA 500ml
 SSP016-NA 1L

Staining Interpretation:
Nuclei: Blue
Keratinized cells: Orange
Superficial cells: Pink
Erythrocytes: Dark pink
Parabasal cells: Greenish blue
Intermediate cells: Greenish blue
Metaplastic cells: Greenish blue/ pink

SUMMARY AND EXPLANATION

For laboratory use only

The Papanicolaou (PAP-NA) stain is used for the cytological examination of exfoliated or aspirated cells. It is commonly used in the study of the morphology of squamous epithelial cells in cervical smears (Pap tests) and other cytology specimens (e.g., body fluids, FNA samples) to detect cellular abnormalities. This product is not intended for diagnostic or therapeutic use. The results are to be interpreted by qualified personnel in conjunction with other clinical and laboratory findings.

PRINCIPLE OF THE PROCEDURE

This technique utilises a combination of dyes in three separate solutions.

- **Hematoxylin:** The basic dye Hematoxylin is the nuclear stain that stains nuclei blue. It has an affinity for the negatively charged sulphate groups of chromatin on the DNA in the nuclei.
- **Orange G6 Solution:** It is the first acid counterstain containing two sulphonic groups due to the phosphotungstic acid-orange G compound. This binds to basic proteins, such as pre-keratin, present in the cytoplasm of keratinised cells. Thus, the cytoplasm of keratinised cells stains orange in different intensities.
- **EA 50 Solution:** It is the second acid counter stain with two dyes, Eosin Y and Light Green SF.
 - Eosin Y is a fluorescent acidic dye that binds to basic compounds like proteins, and stains them from dark red to pink because of the action of bromine on fluorescein. It also stains collagen, muscle fibers, and erythrocytes to pink.
 - Light Green SF is an atmospheric triarylmethane dye with a C2H5N+ reactive group, possessing an affinity for ribonucleic acid of ribosomes, which are abundantly present in pre-keratinised cells. It stains prekeratinized or non-keratinised squamous cells and columnar cells greenish blue.

REAGENTS PROVIDED

Kit Contents		Product Code	Storage temp.	Pack Sizes			
				100ml	250ml	500ml	1L
Harris Hematoxylin (Reagent A)		PS021	RT	100ml	250ml	500ml	1L
Orange G6 (OG-6) Solution (Reagent B) SS001	Orange G6 (OG-6) (Reagent B1)	SS001A	RT	0.3g	0.75g	1.5g	3g
	Phosphotungstic Acid (Reagent B2)	SS002C	RT	0.15ml	0.375 ml	0.75 ml	1.5ml
EA 50 Solution (Reagent C) SS002	Light Green (Reagent C1)	SS002A	RT	1ml	2.5ml	5ml	10ml
	Eosin Y (Reagent C2)	SS002B	RT	2ml	5ml	10ml	20ml
	Phosphotungstic Acid (Reagent C3)	SS002C	RT	2ml	5ml	10ml	20ml
	Glacial Acetic Acid (Reagent C4)	SS002D	RT	2ml	5ml	10ml	20ml

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 the expiration date provided on the vial.**

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

During transport, short-term exposure to temperatures between 2-8 °C does not affect product performance.

SPECIMEN PREPARATION

Recommended positive controls: Gynaecological smears or any superficial cell smears

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. It can cause serious eye and skin irritation. Refer to Materialsheet for any updated risk, hazard or safety information.
4. Dispose of waste observing all local, state, provincial or national regulations.
5. Do not use reagents after expiration date
6. Use protective clothing and gloves, while handling reagents
7. Avoid contamination of reagents as it may lead to incorrect results

MATERIALS REQUIRED, BUT NOT PROVIDED:

- Xylenes
- Graded alcohols (50%, 70%, 80%, 95%, absolute)
- Bluing solution
- Microscopic slides (positively charged)
- Slide holder
- 1% Acid Alcohol

- Cover slips
- Coplin jars
- Methanol
- Distilled water
- Mounting medium

PREPARATION OF WORKING SOLUTION

Important: Refer to the pack size (listed on the box and empty labelled bottle) that received before making any working solutions

Preparation of Orange G6 (OG-6) Working Solution: (Reagent B)

- Measure Absolute Alcohol (check pack size for measuring volume) and pour into an empty labelled bottle provided in the kit, then dissolve Orange G6 (OG-6) (Reagent B1) in absolute alcohol
 - After Orange G (OG-6) is dissolved, add Phosphotungstic acid (Reagent B2) - check pack size for measuring volume and mix well
- NOTE: Any undissolved Orange G (OG-6) after 4 hrs can be discarded. Filter the solution if any artifacts

Components Pack Size	Quantity Required			
	100ml	250ml	500ml	1L
Absolute Alcohol	100ml	250ml	500ml	1L
Orange G6 (OG-6) (Reagent B1)	0.3 gm	0.75g	1.5g	3g
Phosphotungstic Acid (Reagent B2)	0.15ml	0.375ml	0.75ml	1.5ml

Preparation of EA 50 Working solution: (Reagent C)

- Measure Absolute alcohol (check pack size for measuring volume of Absolute Alcohol) into an empty labelled bottle provided in the kit
- Add Light Green (Reagent C1)-check pack size for measuring volume of Light Green and mix thoroughly
- To this, add Eosin Y (Reagent C2)-check pack size for measuring volume of Eosin Y
- Then, add Phosphotungstic Acid (Reagent C3)-check pack size for measuring volume of Phosphotungstic Acid and mix well
- Add Methanol and mix well (check pack size for measuring volume of Methanol)
- Lastly, add Glacial Acetic Acid (Reagent C4)-check pack size for measuring volume of Glacial Acetic Acid
- Make up to the desired volume with absolute alcohol and mix thoroughly.

Components Pack Size	Quantity Required			
	100ml	250ml	500ml	1L
Absolute Alcohol (Not provided)	68ml	170ml	340ml	680ml
Light Green (Reagent C1)	1ml	2.5ml	5ml	10ml
Eosin Y (Reagent C2)	2ml	5ml	10ml	20ml
Methanol (Not provided)	25ml	62.5ml	125ml	250ml
Phosphotungstic Acid (Reagent C3)	2ml	5ml	10ml	20ml
Glacial Acetic Acid (Reagent C4)	2ml	5ml	10ml	20ml

Note: Once the stock reagents are prepared, they remain stable until the expiration date of the kit.

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 an adequate amount of Harris Hematoxylin (Reagent A) to cover the smear completely 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 an adequate amount of Orange G6 (OG-6) Solution (Reagent B) (Refer to the Reagent Preparation) for 3min.
9. Rinse the slide in two changes of 95% alcohol for 30 sec each.
10. Stain the slide with EA 50 Solution (Reagent C) (Refer to the Reagent Preparation) for 10min.
11. Rinse the slide in 95% alcohol for two 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 gynaecological or any superficial cell smears.

PERFORMANCE CHARACTERISTICS

PAP (Papanicolaou) Stain kit (Modified Mayer's Hematoxylin) stains **cell nuclei blue, keratinized cells orange, superficial cells pink, erythrocytes dark pink, parabasal cells greenish blue, intermediate cells greenish blue, and metaplastic cells may stain greenish blue or pink.**

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to the 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. Gently mix all the reagents prior to use.
5. Excessive or incomplete counterstaining may compromise the interpretation of the results.
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. In instances where the staining pattern or localisation differs from the specifications outlined in this datasheet, please get in touch with technical support for guidance.

11. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
12. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
13. This product is intended for single-use application only. Once applied to a tissue section, reagents should not be recovered or reused, as this may compromise test integrity and specificity.
14. PathnSitu makes no warranties beyond those expressly stated in the product description.
15. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
16. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
17. For detailed information and specifications on individual components, please refer to the Product Material Safety Data Sheet (MSDS)

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*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008.127-128.
3. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd edition. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 361-363.

EXPLANATION OF SYMBOLS

LOT

Lot number / Batch number



Expiry



Storage limitation

RT Room Temperature



Date of manufacture

REF

Catalogue number



Manufacturer address