

Papanicolaou Stain for Cytological Preparations - Technical Memo

SOLUTIONS:

	500 ml	1 Gallon	10 Liters
Hematoxylin Stain, Gill I	Part 1180A	Part 1180C	
Hematoxylin Stain, Gill II	Part 1180D	Part 1180F	
Papanicolaou Stain, OG-6	Part 1330A	Part 1330B	Part 1330C
Papanicolaou Stain, EA-36, Fast Green	Part 1300A	Part 1300B	Part 1300C
Papanicolaou Stain, EA-50, Fast Green	Part 1310A	Part 1310B	Part 1310C
Papanicolaou Stain, EA-65, Fast Green	Part 1320A	Part 1320B	Part 1320C
Papanicolaou Stain, EA-36, Light Green	Part 1302A	Part 1302B	Part 1302C
Papanicolaou Stain, EA-50, Light Green	Part 1312A	Part 1312B	Part 1312C
Papanicolaou Stain, EA-65, Light Green	Part 1322A	Part 1322B	Part 1322C

Additionally Needed:

Alcohol, Ethyl Denatured, 95%	Part 10842		
Lithium Carbonate, Saturated Aqueous	Part 12215	or Scott Tap Water Substitute	Part 1380
Alcohol, Ethyl Denatured, 70%	Part 10844		
Alcohol, Ethyl Denatured, 100%	Part 10841		
Xylene, ACS	Part 1445		

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Papanicolaou (Pap) Stain provides three classic staining solutions for cytology preparations, allowing for crisp and distinct nuclear detail and differentially stained cytoplasm. Gill Hematoxylin is the optimal nuclear stain and the use of two counterstains, Orange G (OG) and Eosin Azure (EA) provide the subtle range of green, blue, and pink hues to the cellular cytoplasm.

Papanicolaou Stain, EA, is comprised of a combination of two dyes; Eosin Y and Light Green SF or Fast Green. EA-36, EA-50 and EA-65 denote the varying proportions of dyes in each solution and the formula of choice will depend upon a laboratories staining preference. The Pap Stain, EA also varies with each EA formulation made with either Light Green SF or modified with Fast Green. The choice of which green dye to use will depend upon staining intensity preference.

METHOD:

Fixation/Technique: Choice of fixative and technique is dependent on specimen types analyzed in the laboratory. The stains offered are suitable for both Gyn (EA-36, EA-50) and Non-Gyn (EA-50, EA-65) specimens as determined by the user.

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

- Place slides in 95% Ethyl Alcohol for 10 minutes.
- Rinse in running tap water for 1 minute.
- Stain in Hematoxylin Stain, Gill I or Gill II for 1 to 6 minutes, depending on preference of nuclear stain intensity.
- Wash in tap water until clear.
- Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 30 seconds.
- Wash in tap water for 30 seconds.
- Dehydrate in 70% Ethyl Alcohol; 10 dips.
- Dehydrate in 95% Ethyl Alcohol, 10 dips.
- Stain in Papanicolaou Stain, OG-6 for 30 seconds to 2 minutes, depending on specimen type and preference of stain intensity.
- Rinse in two changes of 95% Ethyl Alcohol; 10 dips each.
- Stain in Papanicolaou Stain, EA-36, EA-50 or EA-65 of choice for 1-3 minutes, depending on specimen type and preference of stain intensity.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Chromatin	Blue
Keratin	Orange
Squamous cells	Shades of pink
Cytoplasm	Shades of blue-green
RBCs, nucleoli, cilia	Shades of pink

PROCEDURE NOTES:

- The Papanicolaou stain solutions can be used for either manual or automated staining. Timings may vary depending on the staining platform used.
- Solutions should be filtered or replaced on a regular basis to prevent cross-contamination.
- Clean and fresh alcohol rinses (Step #10 and Step #12) after the counterstain steps are essential for optimal cytoplasmic staining.
- If using a xylene substitute, closely follow the manufacturer's recommendations for the clearing step.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 127-128.
- Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 361-363.
- Koss, Leopold G. *Diagnostic Cytology and Its Histopathologic Bases*. 3d ed. Philadelphia: Lippincott, 1979. 1218.
- Modifications developed by Newcomer Supply Laboratory.