

# Papanicolaou Staining

## Modified

### For In-Vitro Diagnostics

### For Professional Use Only

Papanicolaou stain is a multichromatic staining cytological technique used to differentiate cells in smear preparations.

#### Sample Preparation

Smears are prepared and fixed immediately with spray fixative (eg Safety Spray) or placed in 95% alcohol for 15 minutes min.

#### Mode of Action

Cells are fixed to a slide, stained with a haematoxylin nuclear stain, then counterstained with a mixture of Eosin Y, Light Green FCF and Orange G.

#### Warnings and Precautions

##### Papanicolaou EA36/50/65

Papanicolaou EA36/50/65 is highly flammable and must be kept away from ignition sources; it is toxic if inhaled or swallowed and harmful in contact with skin. Refer to the Safety Data Sheet



##### Papanicolaou OG6

Papanicolaou OG6 is highly flammable and must be kept away from ignition sources; it can cause serious eye and skin irritation. Refer to the Safety Data Sheet.



#### Ingredients

##### Papanicolaou EA35/50/65

Substance	CAS	Conc
Light Green	5141-20-8	<1%

Eosin Y	17372-87-1	<1%
Phosphotungstic Acid	12501-23-4	<1%
Acetic Acid	64-19-7	<1%
Methanol	67-56-1	<30%
Ethanol	64-17-5	<60%

##### Papanicolaou OG6

Substance	CAS	Conc
Orange G	1936-15-8	<1%
Acetic Acid	64-19-7	<1%
Phosphotungstic Acid	12501-23-4	<1%
Ethanol	64-17-5	>60%
Water	7732-18-5	Balance

#### Specifications

##### Papanicolaou EA36/50/65

Appearance Dark red liquid with greenish tinge  
pH @ 20°C 4.5 – 5.5

##### Papanicolaou OG6

Appearance Bright orange liquid  
pH @ 25°C 3 - 6

#### Stability

The expiry date of each reagent is printed on the label. Store each reagent away in sealed bottle away from heat and light.

This product should not be used if 1) the appearance has changed; 2) the expiration date has passed; or 3) there are other signs of deterioration.

#### Technical Procedure

1. If necessary, remove polyethylene glycol fixative (safety spray) in 50% alcohol, 2 mins
2. Hydrate in 95% alcohol for 2 mins, and 70% alcohol for 2 mins;
3. Rinse in water, 1 min;
4. Stain in Harris Haematoxylin, 5 mins;
5. Rinse in water, 2 min;
6. Differentiate in 0.5% aqueous hydrochloric acid, 10 sec;
7. Rinse in water, 2 min;
8. Blue in Scott's Tap Water substitute, 2 mins;
9. Rinse in water, 2 min;
10. Dehydrate, 70% alcohol, 2 min;
11. Dehydrate 95% alcohol, 2 min;
12. Dehydrate 95% alcohol, 2 min;
13. Stain in OG6, 2 min;
14. Rinse in 95% alcohol, 2min;



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15. Rinse in 95% alcohol, 2 min;
16. Stain in EA50, 3 min;
17. Rinse in 95% alcohol, 1 min;

### Results and Interpretation

Only experienced and suitably qualified persons should carry out interpretation of stained slides.

Nuclei	Blue/Black
Cytoplasm (non-keratinising squamous cells)	Blue/Green
Keratinising Cells	Pink/Orange

### Notes

- Staining times can be adjusted to suit personal preference for lighter or darker staining
- EA65 is recommended for specimens with large amounts of mucus
- Harris Haematoxylin is the optimal nuclear stain, but other aluminium haematoxylin such as Gills can be used.

### References

Survana KS, Layton C, and Bancroft JD. *Bancroft's Theory and Practice of Histological Techniques, 7<sup>th</sup> Ed.* London, Churchill Livingstone, 2012

Sheehan, D.C. and Hrapchak B.B. *Theory and Practice of Histotechnology, 2<sup>nd</sup> Edition; 1987, Battelle Press*

Carson, Freida; Hladick, Christa; *Histotechnology – A Self Instructional Text; 3<sup>rd</sup> Edition; American Society for Clinical Pathology Press 2009*

### Ordering

Product	Size	Code
Papanicolaou EA36	2.5L	FNNII031
Papanicolaou EA50	500mL	FNNII033
Papanicolaou EA50	2.5L	FNNII034
Papanicolaou EA50	5L	FNNII035
Papanicolaou EA65	5L	FNNII038
Papanicolaou OG6	500mL	FNNII039
Papanicolaou OG6	5L	FNNII041
Harris Haematoxylin	500mL	FNNII001
Harris Haematoxylin	2.5L	FNNII002
Scotts Blue Solution	5L	FNNII021