

PRACTICAL-7

H & E Stain with Ehrlich's Hematoxylin.

REGRESSIVE Staining Systems, continue staining..

- Hematoxylin stain is applied
 - Tissue is over stained with hematoxylin
 - Differentiator is used to regressively remove excess hematoxylin
 - Stain procedure continues on with counterstain

H & E Stain with Erhlich's Haematoxylin

It is a general purpose stain or micro-anatomical stain.

HEMATOXYLIN AND EOSIN SOLUTION preparation:

• Hematoxylin _____	1.0 gm
• Distilled water _____	1000ml
• Ammonium or Potassium Alum _____	50gm
• Sodium Iodate _____	0.2gm
• Citric Acid _____	1.0gm
• Chloral Hydrate _____	50gm

METHOD OF PREPARATION:

1. Dissolve Haematoxylin in the water using heat if necessary.
2. Add the Alum by shaking or gentle heat.
3. When the Alum is dissolves add the Sodium Iodate and shake to mix.
4. Add the Citric Acid and gently mix.
5. Add the Chloral Hydrate and shake to dissolve.
6. Bring to boil and continue boiling for 5mins.
7. Cool and it is ready for immediate use. Label recording the date.

• ERHLICH`S HAEMATOXYLIN & EOSIN METHOD

PROCEDURE:

1. Dip slide	Xylene	3mins.
2. Dip Slide	100% alcohol	2min
3. Dip slide	95% alcohol	1min.
4. Dip slide	70% alcohol	1min.
5. Rinse section	Water	
6. Apply	Ehrlich`s haematoxylin	20mins.
7. Rinse in	Tap water again	2mins.
8. Differentiate	1% acid alcohol	1 to 2dips
9. Wash in	Water	
10. Blue in	Ammonia water	1min.
11. Wash in	Tap water	
12. Apply	Eosin	3mins.
13. Apply	70% alcohol	30seconds
14. Apply	95% alcohol	10seconds
15. Dehydrate	100% alcohol	10seconds
16. Dehydrate	100% alcohol	15seconds
17. Clear in 2 changes	Xylene	2mins.
	Xylene	2mins.
18. Mount in	DPX-cover slip ping	

- **ERHLICH`S HAEMATOXYLIN & EOSIN METHOD**

RESULT: Cell Nuclei _____ **blue**

RBCs _____ **red**

Connective tissue & cell cytoplasm ____ **shade of pink**

