

# **Protocol Booklet**

Product Code(s) HB6189

HB0758

Product Name(s) Modified Mayer's Hematoxylin

Eosin Y

Purpose H&E Staining of Paraffin Embedded Tissue Sections

**Please note**: This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use



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#### **Product Overview**

Hematoxylin and eosin (H&E) staining is a commonly used technique in histology to visualize tissue structure under a microscope. Hematoxylin stains cell nuclei blue or purple, while eosin stains the cytoplasm and extracellular matrix pink, creating contrast that highlights cellular details. It is widely used in pathology to help diagnose diseases, including cancer, by revealing tissue architecture and abnormalities.

#### **Components & Storage**

Required stains:

SKU	Component	Quantity	Storage Temperature
HB0758	Eosin Y	1g	4°C
HB6189	Modified Mayer's Hematoxylin	250ml	RT

This protocol additionally requires the below. The volume of reagent required will depend upon the size of the staining jars used.

Component	Quantity	Storage Temperature
Ethanol	≈1L	RT
Histoclear	≈250ml	RT
Xylene	≈1L	RT
DPX	≈20ml	RT
Scott's Water*	≈1L	RT
Acid Alcohol <sup>*</sup>	≈1L	RT

<sup>\*</sup> Please see recipe at the end of this protocol book.

#### **Protocol**

This protocol is based upon staining of paraffin embedded tissue sections. For information on fixing and embedding of sections please refer to our <u>Paraffin Embedded IHC Protocol (IHC-P)</u>.

- 1. Incubate paraffin embedded sections in histoclear for 20 minutes
- 2. Incubate slides in 100% ethanol for 3 minutes
- 3. Incubate slides in 90% ethanol for 3 minutes
- 4. Incubate slides in 70% ethanol for 3 minutes
- 5. Rinse in tap water
- 6. Incubate in hematoxylin solution for 5 minutes
- 7. Rinse in running tap water for 5 minutes
- 8. Dip in acid alcohol (8-12 fast dips)
- 9. Incubate slides in tap water for 2 minutes
- 10. Incubate slides in Scott's water for 2 minutes
- 11. Incubate slides in tap water for 2 minutes
- 12. Incubate slides in Eosin working solution for 30-60s
- 13. Dip and swish in 70% ethanol
- 14. Dip and swish in 90% ethanol
- 15. Incubate slides in 100% ethanol for 3 minutes
- 16. Incubate slides in three changes of xylene for 1 minute per incubation
  - a. Carry out in a fume hood as xylene is extremely toxic
- 17. Mount with DPX and leave to dry in a fume hood overnight before imaging



### **Recipes**

#### 1% Eosin Stock

Reagent	Quantity
Eosin Y (HB0758)	1g
Ethanol	80ml
dH <sub>2</sub> O	20ml

Please note: Store at room temperature.

#### **Eosin Working Solution**

Combine all ingredients and filter before use.

Reagent	Amount to add			Final concentration	
J	150ml	300ml	600ml		
1% Eosin Stock	45ml	90ml	180ml	0.3%	
Ethanol	108ml	216ml	432ml	80%	
dH <sub>2</sub> O	27ml	54ml	108ml	20%	
Glacial Acetic Acid	900µl	1.8ml	3.6ml	-	

Please note: Store at room temperature.

#### **Ethanol**

Combine all ingredients.

	Ethanol Concentration				
	70% 90% 100%				
Ethanol	700ml	900ml	1000ml		
dH <sub>2</sub> O	300ml	100ml	0ml		

Please note: Store at room temperature in a flammables cabinet.

#### **Acid Alcohol**

Combine all ingredients.

Reagent	Amount to add			d Final concentration	
	50ml	250ml	1000ml		
Ethanol	35ml	175ml	700ml	70%	
dH <sub>2</sub> O	15ml	75ml	300ml	30%	
10M HCI	125µl	625µl	2.5ml	25mM	

Please note: Store at room temperature



#### **Scott's Water**

Combine all ingredients.

Reagent	Amount to add			Final concentration
3.3	50ml	250ml	1000ml	
MgSO <sub>4</sub>	0.5g	2.5g	10g	83mM
NaHCO <sub>3</sub>	0.033g	0.165g	0.66g	7.85mM
dH <sub>2</sub> O	50ml	250ml	1000ml	-

Please note: Store at room temperature

## Guidelines, precautions, troubleshooting

Please contact our technical support team at <u>technicalhelp@hellobio.com</u> for advice on how to resolve any problems encountered when using this product. Observe safe laboratory practice and consult the safety datasheet. Please see the datasheet on our website for general guidelines, precautions, limitations on the use of the product.

#### Contact

For customers in the UK, Europe and Rest of the World

Customer Care <a href="mailto:customercare@hellobio.com">customercare@hellobio.com</a>

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