

SOP  
#1

Subject **Regressive H&E staining on frozen sections**

Sheet **1** of **2**

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| Rev 1   | Effective Date | Author  |
|---------|----------------|---------|
| 6/12/18 | 8/20/15        | Fimbres |

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### 1. PURPOSE

The purpose of an H&E is to differentiate between purple nucleic acids (such as nucleus of cells) and pink proteins.

### 2. SCOPE

This standard operating procedure applies to all frozen sections brought to TACMASR requiring H&E staining.

### 3. RESPONSIBILITIES

The person carrying out this protocol should know how to operate the autostainer and have knowledge on the outcome of the tissue as per his/her pathologist prefers.

### 4. MATERIALS and EQUIPMENT

Leica Autostainer XL  
70% ethanol  
95% ethanol  
2x 100% ethanol  
3x xylene  
Coverslips  
Mounting media  
Gloves  
Hematoxylin (Leica/Fisher - 3801575)  
Eosin (Leica – NC9003835)  
Clearview (Leica – 3803598)  
Bluing Reagent (leica – 3802918)

### 5. SAFETY AND CAUTIONARY NOTES

### 6. PROCEDURE

1. Frozen sections cut at 5um
2. Fixed in chilled acetone for 3 minutes
3. Hematoxylin for 2 minutes
4. Running water for 30 seconds
5. Clearview for 30 seconds
6. Running water for 30 seconds
7. Bluing for 30 seconds

8. Running water for 30 seconds
9. 70% ethanol for 1 minute
10. Eosin for 2 minutes
11. 100% ethanol for 1 minute
12. 100% ethanol for 1 minute
13. 100% ethanol for 1 minute
14. Xylene for 1 minute
15. Xylene for 1 minute
16. Xylene for 1 minute