# Movat 五色法染色实验报告

## 一、实验器材及试剂

1、 实验器材

| 名称 | 厂家 | 型号 |
|---|---|---|
| 脱水机 | DIAPATH | Donatello |
| 包埋机 | 武汉俊杰电子有限公司 | JB-P5 |
| 病理切片机 | 上海徕卡仪器有限公司 | RM2016 |
| 冻台 | 武汉俊杰电子有限公司 | JB-L5 |
| 组织摊片机 | 浙江省金华市科迪仪器设备有限公司 | KD-P |
| 烤箱 | 天津市莱玻瑞仪器设备有限公司 | GFL-230 |
| 载玻片 | Wanwu | |
| 正置光学显微镜 | 日本尼康 | NIKON ECLIPSE E100 |
| 成像系统 | 日本尼康 | NIKON DS-U3 |

2、 主要实验试剂

| 试剂名称 | 厂家 | 货号 |
|---|---|---|
| 无水乙醇 | 国药集团化学试剂有限公司 | 100092683 |
| 二甲苯 | 国药集团化学试剂有限公司 | 10023418 |
| 氨水 | 国药集团化学试剂有限公司 | 10002118 |
| 天狼猩红 | Wanwu | G1018-500ML |
| 中性树胶 | 国药集团化学试剂有限公司 | 10004160 |
| 酸性品红 | 国药集团化学试剂有限公司 | 71019360 |
| 冰醋酸 | 国药集团化学试剂有限公司 | 10000218 |
| 磷钼酸 | 国药集团化学试剂有限公司 | 20029916 |
| 阿利新蓝 | Wanwu | G1027-100ML |
| 番红 O | Solarbio | S8020 |
| EVG 染液套装 | Wanwu | G1042 |

## 二、试剂配制

碱性乙醇：95%乙醇配制成 3%氨水溶液；
番红 O 储备液：1.0g 番红 O 粉剂溶于 100mL 纯水
酸性品红储备液：取 0.1g 酸性品红加入 99.5mL 纯水，再加入 0.5mL 冰醋酸，搅拌充分溶解；
番红/酸性品红工作液：每 50mL 工作液内含番红 0 储备液 40mL 和酸性品红储备液 10mL；
1%磷钼酸：磷钼酸 1g，纯水定容至 100mL
1%冰醋酸：冰醋酸 1mL，纯水定容至 100mL

## 三、实验步骤

**1**、石蜡切片脱蜡至水：依次将切片放入二甲苯I20min-二甲苯II20min-无水乙醇I5min-无水乙醇II5min-75%酒精 5min，自来水洗。
**2**、阿利新蓝染色：切片入 1%阿利新蓝溶液染色 15min；
**3**、分化：切片入提前在 60℃预热 30min 的碱性乙醇中 3-5s，自来水洗，镜检至阳性部位呈蓝色背景近乎无色即可，或者无阳性时，组织无蓝色着色；
**4**、EVG 染色：组画笔画圈圈住组织，滴加 EVG 染色液染色 5min，用盛装自来水的洗瓶轻轻冲洗组织，洗净染液，切片入 5%$FeCl_3$溶液中快速分化 2s，自来水洗终止分化，可反复分化水洗和镜检，至弹力纤维呈黑色且清晰，背景近无色。
**5**、番红 O/酸性品红染色：切片入番红 O/酸性品红工作液（现配现用，不可保存）染色 4min，自来水洗 10s；
**6**、分化：1%磷钼酸染 2min；切片不水洗直接入 1%的冰醋酸分化 4min；
**7**、天狼猩红染色：自来水稍洗 10s，切片入天狼猩红染液染色 2min；
**8**、切片依次入 3 缸无水乙醇快速脱水，各 3s、5s、1min，二甲苯透明 5min，中性树胶封片。
**9**、显微镜镜检，图像采集分析。

## 三、结果判读

细胞核和弹力纤维呈黑色，蛋白聚糖呈蓝色，胶原纤维呈红色，肌纤维呈红色，泡沫细胞淡紫色。

## 四、注意事项

EVG 分化时需要镜检控制染色背景和分化程度，尤其是冰冻切片，只有控制好才能保证后续颜色不叠加或者少量叠加。

# Movat staining experimental report

## 1. Experimental equipment and reagents

1.1 Experimental equipment

| Name | Manufactor | Model |
|---|---|---|
| Dehydrator | DIAPATH | Donatello |
| Embedding machine | Wuhan Junjie Electronics Co., Ltd | JB-P5 |
| Pathological section machine | Shanghai Leica Instrument Co., Ltd | RM2016 |
| Frozen platform | Wuhan Junjie Electronics Co., Ltd | JB-L5 |
| KD-P Water Bath | Kedee | KD-P |
| Oven | Tianjin Lai Bo Rui Instrument Equipment Co., Ltd | GFL-230 |
| Slides | Wanwu | |
| Orthostatic microscope | NIKON, JAPAN | NIKON ECLIPSE E100 |
| Image system | NIKON, JAPAN | NIKON DS-U3 |

1.2 Main experimental reagents

| Reagent name | Manufactor | Article number |
|---|---|---|
| Absolute ethanol | Sinopharm Group Chemical Reagent Co. LTD | 100092683 |
| Xylene | Sinopharm Group Chemical Reagent Co. LTD | 10023418 |
| Aqueous ammonia | Sinopharm Group Chemical Reagent Co. LTD | 10002118 |
| Sirius Scarlet | Wanwu | G1018-500ML |
| Neutral resin | Sinopharm Group Chemical Reagent Co. LTD | 10004160 |
| Acid magenta | Sinopharm Group Chemical Reagent Co. LTD | 71019360 |
| Glacial acetic acid | Sinopharm Group Chemical Reagent Co. LTD | 10000218 |
| Phosphomolybdic acid | Sinopharm Group Chemical Reagent Co. LTD | 20029916 |
| Alcian blue | Wanwu | G1027-100ML |
| Safranin O | Solarbio | S8020 |
| EVG dye solution kit | Wanwu | G1042 |

## 2. Reagent preparation

Alkaline ethanol: 95% ethanol is formulated into 3% ammonia solution.
Safranin O solution: 1.0 g Safranin O powder dissolved in 100 mL pure water.
Acid magenta solution: 0.1g acid magenta dissolved in 99.5mL pure water, then added 0.5mL glacial acetic acid, stirred to dissolve fully.
Safranine/acid magenta solution: Every 50mL working solution contains 40mL safranine O solution and 10mL acid fuchsia solution.
1% Phosphomolybdic acid: 1g phosphomolybdic acid dissolved in 100mL pure water.
1% Glacial acetic acid: 1mL glacial acetic acid dissolved in 100mL pure water.

## 3. Experimental steps

**3.1** Paraffin slides dewaxed as follow: Two changes of pure xylene for 20 min. Two changes of pure ethanol for 5min. 75% ethanol for 5min. Keep slides in tap water.
**3.2** Alcian blue staining: The slides were stained in 1% Alcian blue solution for 15min.
**3.3** Heating the alkaline ethanol in advance for 30min to 60℃. After immersing the slides in alkaline ethanol for 3-5s, wash with tap water. Observed under microscopic shows that the positive part is blue and the background is almost colorless. Or when there is no positive, the tissue is not blue.
**3.4** EVG staining: Draw a circle around the tissues with Pap-Pen, add EVG dye solution for 5 min. Rinse the tissue gently with a wash bottle containing tap water until the stain was washed away. The slides were put into 5% $FeCl_3$ solution for rapid differentiation for 2 s, and washed with tap water to terminate differentiation. It can be repeatedly differentiated, washed and observed under microscopically, until the elastic fibers were black and clear, and the background was nearly colorless.
**3.5** Saffron O/acid fuchsin staining: The slides were stained with Safranin O/Acid magenta solution (Do not save) for 4min and washed with tap water for 10s.
**3.6** Stained with 1% phosphomolybdic acid for 2 min, and the slides were directly washed with 1% glacial acetic acid for 4 min.
**3.7** Sirius scarlet staining: Wash with tap water for 10s, and put the slide into the Sirius Scarlet Stain for 2min.
**3.8** The slides were successively dehydrated into 3 cylinders of absolute ethanol for 3s, 5s, 1min. Immersed in xylene to transparent and sealed with neutral resin.
**3.9** Observed under microscope and took images..

## 4. The results were as follows

The nuclei and elastic fibers were black, the proteoglycan was blue, the collagen fibers and muscle fibers were red and the foam cells were lilac.

**5. Precautions**

During EVG differentiation, microscopic examination is required to control the staining background and the degree of differentiation, especially for frozen sections. Only by controlling well can the subsequent colors not overlap or overlap slightly.