



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	Servicebio	
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	Servicebio	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	Servicebio	G1027-100ML
番红 O	Solarbio	S8020
EVG 染液套装	Servicebio	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 O 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%FeCl₃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	Servicebio	
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	Servicebio	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	Servicebio	G1027-100ML
Safranin O	Solarbio	S8020
EVG dye solution kit	Servicebio	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.

Safranin/acid magenta solution: Every 50mL working solution contains 40mL safranin 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°C. 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% FeCl₃ 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



武汉赛维尔生物科技有限公司

Wuhan Servicebio Technology Co., Ltd.

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.