EVG 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	G6004
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
EVG 染液套装	Wanwu	G1042
中性树胶	国药集团化学试剂有限公司	10004160

二、实验步骤

1、石蜡切片脱蜡至水：依次将切片放入二甲苯I 20min-二甲苯II 20min-无水乙醇I 5min-无水乙醇II 5min-75%酒精 5min，自来水洗。

2、EVG 染色：EVG 染液 A：EVG 染液 B：EVG 染液 C5：2：2 混合成 EVG 染液（提前两天配置），切片入 EVG 染液染 5min，自来水冲洗。

3、背景分化：EVG 染液 B 稀释一倍后稍分化一下，自来水洗一下，如此反复操作，在显微镜下控制分化程度，至弹力纤维呈紫黑色，背景呈灰白色近无色。

4、复染 VG：EVG 染液 E 9ml 加入 EVG 染液 D 1ml 混合成 VG 染液（按比例配制用多少配

多少），染 1-3min（染色时间视组织中弹力纤维成分而定，染色时间过短胶原颜色浅，染色时间过长弹力纤维会褪色），快速水洗，无水乙醇三缸快速脱水。

5、透明封片：两缸干净的二甲苯透明各 20s、5min（二甲苯专用不与其他二甲苯共用），中性树胶湿封。

6、显微镜镜检，图像采集分析。

三、结果判读：

弹性纤维呈紫黑色，胶原纤维为红色，背景为黄色。

四、注意事项：

分化时，至弹性纤维呈紫黑色的细丝状即可，不可过度分化，弹性纤维褪色，若分化不足，则VG复染后效果不佳。

EVG staining report

1 Apparatus and reagents

1.1 Major apparatus

Name	Producer	Model
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan Junjie Electronics Co., Ltd	JB-P5
Pathology slicer	Leica	RM2016
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Organizer	KEDEE	KD-P
oven	Labotery	GFL-230
Glass slide	Wanwu	G6004
Upright optical microscope	Nikon	NIKON ECLIPSE E100
Imaging system	Nikon	NIKON DS-U3

1.2 Major reagents

Name	Producer	Code
Ethanol	SCRC	100092683
Xylene	SCRC	10023418
EVG dye solution set	Wanwu	G1042
Neutral gum	SCRC	10004160

2 Procedure

2.1 Dewaxing as followed:

Xylene I for 20 min;

Xylene II for 20 min;

100% ethanol I for 5 min;

100% ethanol II for 5 min;

75% ethanol for 5 min;

Rinsing with tap water ;

2.2 EVG A, EVG B and EVG C were prepared into EVG solution according 2 days in advance to the ratio of 5:2:2. Then stain with EVG B for 5 min, rinsing with tap water.

2.3 Treat the section repeatedly with double diluted EVG B and tap water until the background was separated. Control the degree of differentiation under a microscope, until the elastic fibers are purple-black, and the background is gray-white and almost colorless.

2.4 VG dye solution was prepared according to the ratio of 9ml EVG E and 1ml EVG D. Configure according to actual usage. Stain with VG solution for 1-3 min. Then washing it fast with water, dehydration quickly with three cup of anhydrous ethanol.It should be noted that the staining time depends on the composition of the elastic fibers in the tissue. If the staining time is too short, the collagen will be light in color, and if the staining time is too long, the elastic fibers will fade.

2.5 Xylene I for 20 s; Xylene II for 5 min; Finally seal with neutral gum.Please do not use xylene used in other experiments.

2.6 Observe with microscope inspection, image acquisition and analysis.

3 Results

Color	Result
Purple-black	Elastic fibers
Red	Collagen fibers
Yellow	Background

4 Precautions

4.1 During the differentiation, the elastic fibers should be in the form of purple-black filaments. Do not over-differentiate, otherwise the elastic fibers will fade. If the differentiation is insufficient, the effect of VG counterstaining will be poor.