天狼星红染色实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
脱水机	常州市中威电子仪器有限公司	TSJ-SD
包埋机	常州市中威电子仪器有限公司	BMJ-A
病理切片机	赛默飞世尔科技有限公司	SHANDON FINESSE 325
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	常州市中威电子仪器有限公司	PHY-III
防脱载玻片	湖北百奥斯生物科技有限公司	BP0510
正置显微镜	奥林巴斯有限公司	CX-31
成像系统	日本滨松光子学株式会社	NanoZoomer®S360

2、主要试剂及货号

名称	厂家	型号
无水乙醇	国药集团化学试剂有限公司	100092683
环保透明剂	同声科技	
环保封片剂	同声科技	
天狼星红染液	湖北百奥斯生物科技有限公司	

二、实验步骤

1、中性甲醛液固定组织，石蜡切片常规脱蜡至水。

2、入天青石蓝液染 5-10 分钟，蒸馏水洗。（也可用 Harris 苏木素代替）

3、用天狼星红饱和苦味酸液染 15-30 分钟。

4、无水乙醇直接分化与脱水，大约要四缸进行脱水。

5、二甲苯透明，中性树胶封固，镜检。

三、结果判读

胶原纤维呈红色，细胞核呈绿色，其他呈黄色。

四、注意事项

1、及时用偏振光显微镜进行观察与拍照，以保持鲜艳的色彩。也可用白光显微镜观察，但只能看到红、黄两种颜色。

2、在偏振光显微镜下可以观察到四种类型的胶原纤维：

（1）Ⅰ型胶原纤维：紧密排列，显示很强的双折光性，呈黄色或红色的纤维。

（2）Ⅱ型胶原纤维：显示弱的双折光性，呈多种色彩的疏松网状分布。

（3）Ⅲ型胶原纤维：显示弱的双折光性，呈绿色的细纤维。

（4）Ⅳ型胶原纤维：显示弱的双折光基膜，呈淡黄色。

Experimental Report on Picro Sirius Red Staining

1. Instruments and key reagents

1.1 Instruments

Instrument	Manufacture	Specifications/Model
Tissue processor	Changzhou Zhongwei Electronics Co., Ltd	TSJ-SD
Tissue embedder	Changzhou Zhongwei Electronics Co., Ltd	BMJ-A
Microtome	ThermoFisher Scientific	SHANDON FINESSE 325
Freezing table	Wuhan Junjie Electronics Co., Ltd	JB-L5
Water bath - Slide drier	Changzhou Zhongwei Electronics Co., Ltd	PHY-III
Slide	Hubei BIOSSCI Biotech Co., Ltd	BP0510
Upright microscope	Olympus	CX-31
Digital scanner	HAMAMATSU PHOTONICS	NanoZoomer®S360

1.2 Key reagents

Reagent	Manufacture	Specifications/Model
Ethanol	Sinopharm	100092683
Clearer	Wuhan Tongsheng Technology Development Co., Ltd	
Neutral balsam	Wuhan Tongsheng Technology Development Co., Ltd	
Picro Sirius Red staining kit	Hubei BIOSSCI Biotech Co., Ltd	

2. Procedures

2.1 Tissues were fixed with neutral formaldehyde fix solution.

2.2 Deparaffinization and rehydration.

2.2.1 Tissue sections were immersed in clearer for 10min. Repeat this step two times, gently shaking off excess liquid between each step.

2.2.2 Tissue sections were immersed in progressively more dilute ethanol solutions and ultimately immersed in distilled water to rehydrate the tissue: Absolute ethanol for 5min, 95% ethanol for 5min, 85% ethanol for 5min, 75% ethanol for 5min. Rinsing with distilled water for 1min.

2.3 The tissue sections were stained with celestine blue solution for 5-10 minutes and washed with distilled water. (Harris hematoxylin can also be used instead)

2.4 The tissue sections were stained with saturated Picro Sirius Red solution for 15-30 minutes.

2.5 The tissue sections were differentiated and dehydrated with absolute ethanol, which required about four cylinders of ethanol for dehydration.

2.6 The tissue sections were transparent with xylene and mounted with neutral balsam.

3. Results

Collagen fibers are red. Nuclei are green and the others are yellow.

4. Attentions

4.1 It is crucial to observe and take photos with a polarization microscope in time to keep bright colors. It can also be observed under an upright microscope, but only red and yellow can be seen.

4.2 There are four types of collagen fibers can be observed under a polarization microscope:

Collagen I: Yellow or red fibers. Collagen fibers are tightly arranged, showing strong birefringence.

Collagen II: It shows weak birefringence and a loose network distribution with

various colors.

Collagen III: It shows weak birefringence and is a green fine fiber.

Collagen IV: It shows basal lamina with weak birefringence, which is light yellow.

5. Technical support

The technical support of Picro Sirius Red staining experiment is provided by Hubei BIOSSCI Biotech Co., Ltd (Wuhan Changyan Pathology technology Co., Ltd).

Tel: 400 118 0100

Fax: +86-027-87382710

E-mail: support@biossci.com

Website: www.biossci.com