植物硬组织切片甲苯胺蓝染色实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
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
甲苯胺蓝染液	Servicebio	G1032
冰醋酸	Servicebio	G10000218
中性树胶	国药集团化学试剂有限公司	10004160
乙二醇乙醚乙酸酯	麦克林	E808814-2.5L

二、实验步骤

1、切片脱塑至水：依次将切片乙二醇乙醚乙酸酯I 6 h，37℃，乙二醇乙醚乙酸酯II过夜，37℃，乙二醇乙醚乙酸酯III室温 10-15 min，乙二醇乙醚乙酸酯IV室温 10-15 min，100%I乙醇 10 min，100%II乙醇 10 min，95%乙醇 10 min，90%乙醇 10 min，80%乙醇 10 min，自来水洗。

2、甲苯胺蓝染色：植物组织切片入染液约 2 min 后水洗，镜检，根据组织着色深浅进行适当的分化或者不分化，自来水洗后，将切片置于 60℃烤箱内烤干。

3、透明封片：切片入干净的二甲苯透明 5 min，中性树胶封片。

4、显微镜镜检，图像采集分析。

三、结果判读：

木质化细胞壁呈蓝绿色，纤维素细胞壁呈紫蓝色。

四、注意事项：

1、染完后的切片不能长时间放在水里，会褪色。

2、组织一定要完全烤干后再封片，避免组织残留小水珠。

Hard Plant Toluidine Blue Stain Report

1. Experimental equipment and reagents

1.1 Equipment

Brand name	Manufactures	Model
Oven	Tianjin Labotery instrument equipment Co. Ltd	GFL-230
Microslide	Servicebio	
Optical Microscopy	NIKON	Nikon Eclipse E100
Imaging system	NIKON	Nikon DS-U3

1.2 Reagent

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylene	Sinopharm Chemical Reagent Co.,Ltd	100023418
Toluidine blue staining solution	Servicebio	G1032
Glacial acetic acid	Servicebio	G10000218
Neutral balsam	Sinopharm Chemical Reagent Co.,Ltd	10004160
Ethylene glycol ethyl ether acetate	Macklin	E808814-2.5L

2. Experiment procedure

2.1 Remove the embedding agent and rehydration

Put sections into ethylene glycol ethyl ether acetate I 6 h at 37 ℃ and ethylene ether acetate II overnight at 37 ℃. Then put them into ethylene glycol ethyl ether acetate III 10-15 minutes at room temperature and ethylene glycol ethyl ether acetateIV 10-15 minutes at room temperature. The sections were rehydrated in 100%I% - 100%II - 95% - 90% - 80% alcohol. Each step takes anywhere for 10 minutes. Finally, rinse with running water.

2.2 Toluidine blue stain

The sections were put into the toluidine blue staining solution for about 2 minutes, then washed and observed under inverted microscope. The sections were differentiated or undifferentiated

according to the color depth of the tissue. The sections were placed in an oven at 60℃ for drying after washed with running water.

2.3 Transparent and mount

Put sections into three cylinders of xylene for 5 min. Finally, mount the sections with neutral balsam.

2.4 Microscope observation and collection and analysis images.

3. Result determination

The cytoderm which has lignified appear glaucous and the color of cellulose cell wall is bluish violet.

4. Matter need attention

4.1 After stain, the sections should not be kept in the water for a long time because the color will fade in the water.

4.2 Tissue must be completely dried and then mount the sections with neutral balsam.