甲苯胺蓝染色（尼氏小体）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	
甲苯胺蓝染液	湖北百奥斯生物科技有限公司	BP0360

二、实验步骤

1、石蜡切片脱蜡至水：依次于环保脱蜡剂(1)、环保脱蜡剂(2)、环保脱蜡剂(3)中分别脱蜡 10 分钟，然后经无水乙醇、95%乙醇、85%乙醇、75%乙醇各 5 分钟。蒸馏水洗 3 次。

2、切片置于预热 50℃ 1%甲苯胺蓝水溶液中，并于 56℃温箱中染 20min，蒸馏水洗干净。

3、95%酒精或 0.1%冰醋酸分化，镜下控制，以尼氏小体显示清晰为度。

4、无水乙醇迅速脱水。

5、环保透明剂透明、封片、镜检。

三、结果判读

细胞核呈淡蓝色，尼氏小体呈深蓝色，背景无色或呈浅蓝色。

四、注意事项

1、尼氏体离体后容易溶解，因此组织取出后应立即固定，否则难以着色。

2、95%乙醇分化应迅速进行，肉眼观察至切片清晰，背景呈淡蓝色或无色为适宜。

Experimental Report on Toluidine Blue (Nissl Bodies) 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	
Toluidine blue solution	Hubei BIOSSCI Biotech Co., Ltd	BP0360

2. Procedures

2.1 Deparaffinization and rehydration.

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

2.1.2 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.

2.2 Tissue sections were placed in preheated (50℃), 1% toluidine blue aqueous solution and sections were stained in incubator at 56℃ for 20 minutes. Then, sections were washed with distilled water.

2.3 Tissue sections were differentiated with 95% ethanol or 0.1% glacial acetic acid and controlled under microscope until Nissl bodies were clearly visible.

2.4 Tissue sections were dehydrated rapidly with absolute ethanol.

2.5 Tissue sections were put into clearer and then mounted with neutral balsam. Examination with microscope.

3. Results

Nuclei are pare blue. Nissl bodies are dark blue. The background is light blue or colorless.

4. Attentions

4.1 Tissues should be fixed immediately because the Nissl body is easy to dissolve in vitro, otherwise, it is difficult to be stained.

4.2 The differentiation with 95% ethanol should be carried out quickly. Observe the tissue section with naked eyes until the section is clear and the background is light blue or colorless.

5. Technical support

湖北百奥斯（Biossci）生物科技有限公司
武汉长衍病理科技有限公司

The technical support of Toluidine Blue (Nissl Bodies) staining experiment is provided by Hubei BIOSSCI Biotech Co., Ltd (Wuhan Changyan Pathology technology Co., Ltd).

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