高尔基染色（golgi-cox）实验报告

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
冰冻切片机	Thermo	CRYOSTAR NX50
切片刀	上海徕卡仪器有限公司	LEICA 819
载玻片	江苏世泰实验器材有限公司	10127105P-G
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
数字切片扫描仪	D HISTECH	Pannoramic 250

2、主要实验试剂

试剂名称	厂家	货号
固定液	Wanwu	G1101
OCT 包埋剂	Sakura	4583
高尔基染液套装	Wanwu	G1069
氨水	国药集团化学试剂有限公司	10002118
甘油明胶	Wanwu	G1402
冰醋酸	国药集团化学试剂有限公司	10000218
无水乙醇	国药集团化学试剂有限公司	100092683
蔗糖	国药集团化学试剂有限公司	57-50-1
明胶硫酸铬钾混合液	Wanwu	

二、实验步骤

明胶硫酸铬钾混合液(放置 4℃条件下可保存 3 个月)

明胶玻片制作：将明胶硫酸铬钾混合液至于 37℃加热呈液体状(不能摇动该溶液，溶液中若形成小气泡，后续制片过程中会造成玻片上残留有很多小颗粒)，普片浸入该液体几秒后拿出，置于铁架上于 37℃烤箱烘干即可，干燥阴凉通风处保存。

1、取材：将老鼠杀死后，立刻取脑组织置于固定液中固定 48h 以上。

2、高尔基染色：根据需要观察的组织部位将大鼠脑组织切成 2-3mm 厚的组织块，用生理盐水将脑组织轻轻漂洗几遍，置于 45ml 的圆底 EP 管中，加入高尔基染液将脑组织完全浸没，

放置阴凉通风处避光处理 14 天（浸泡 48h 后，换一次新染液，之后每隔 3 天换一次新染液，共计 14 天）。

3、组织处理：蒸馏水浸洗 3 次，倒入 80%的冰醋酸浸没组织，过夜，待组织变软后蒸馏水洗，置于 30%的蔗糖中。

4、切片：用振荡切片机将组织切成 100 微米，贴在明胶玻片上，过夜避光晾干。

5、显影定影：将晾干后的组织玻片以浓氨水处理 15min，蒸馏水洗 1min，酸性坚膜定影液处理 15min，蒸馏水洗 3min，晾干，甘油明胶封片。

6、显微镜镜检，图像采集分析；可用数字切片扫描仪全景多层扫描得到脑组织全景图像。

三、结果判读：

神经元呈黑色，胶质细胞呈黑色，背景灰白色或无色。

四、注意事项：

1、高尔基染液有剧毒，需要穿戴实验服和一次性手套操作实验；

2、染色过程中的每一步都需要严格避光。

3、高尔基染液放置阴凉通风避光处常温保存即可；定影液18℃-20℃避光通风处保存。

Golgi-cox Staining Experiment Report

I. Experimental equipment and reagents

1. Experimental equipment

Equipment name	Manufacturer	Model No.
Freezing microtome	Thermo	CRYOSTAR NX50
Slicing knife	Shanghai Leica Instrument Co., Ltd.	LEICA 819
Microscope slide	Citotest Labware Manufacturing Co.，Ltd.	10127105P-G
Upright electron microscope	JAPAN NIKON	NIKON ECLIPSE E100
Imaging system	JAPAN NIKON	Nikon DS-U3
Digital slide scanner	3D HISTECH	Pannoramic 250

2. Main experiment reagents

Reagent name	Manufacturer	Item No.
Fixative	Wanwu	G1101
OCT Embedding medium	Sakura	4583
Golgi-cox staining solution kit	Wanwu	G1069
Ammonium hydroxide	Sinopharm Chemical Reagent Co., Ltd.	10002118
Glycerin gelatin	Wanwu	G1402
Glacial acetic acid	Sinopharm Chemical Reagent Co., Ltd.	10000218
Anhydrous ethanol	Sinopharm Chemical Reagent Co., Ltd.	100092683
Sucrose	Sinopharm Chemical Reagent Co., Ltd.	57-50-1
Gelatin potassium chromium sulfate mixture	Wanwu	

II. Experiment steps

Gelatin potassium chromium sulfate mixture (It can be stored for 3 months under 4℃ condition.)

Production of gelatin slides: Heat the gelatin potassium chromium sulfate mixture to liquid at 37℃ (do not shake the solution, if small bubbles are formed in the solution, many small particles will remain on the slide during the subsequent production process), immerse ordinary slides into the liquid for a few seconds and take out, place on an iron rack and dry in a 37℃ oven, store in a dry, cool and ventilated place.

1. Draw materials: After killing the rat, immediately take the brain tissue and fix it in the fixative for more than 48h.

2. Golgi-cox staining: Cut the rat brain tissue into tissue blocks with thickness of 2-3mm according to the tissue site to be observed, gently rinse the brain tissue with normal saline for several times, then place in a 45ml round bottom EP tube, add Golgi-cox staining solution to completely submerge the brain tissue, and place in a cool and ventilated place and avoid light to treat for 14 days (after 48h soak, change the new staining solution, and then change the new staining solution every 3 days, total of 14 days).

3. Tissue treatment: Immerse in distilled water for 3 times, pour in 80% glacial acetic acid to immerse the tissue overnight, wait for the tissue to become soft, then wash with distilled water, and place into 30% sucrose.

4. Sectioning: Cut the tissue into 100 microns with oscillating microtome, paste it on a gelatin slide, and dry in the dark overnight.

5. Developing and fixing: Treat dried tissue slides with concentrated ammonia water for 15 minutes, wash with distilled water for 1 minute, treat with acid hardening fixing solution for 15 minutes, wash with distilled water for 3 minute, dry and seal the section with glycerin gelatin.

6. Microscope inspection, image acquisition and analysis, obtain panoramic images of brain tissue by panoramic multi-layer scanning with digital slice scanner.

III. Interpretation of results:

Neurons are black, glial cells are black, and the background is grayish white or colorless.

IV. Precautions:

1. The Golgi-cox staining solution is highly toxic, so the laboratory staff need to wear lab coat and disposable gloves to perform the experiment.
2. Each step during staining needs to be strictly protected from light.
3. The Golgi-cox staining solution should be stored in a cool, ventilated and dark place at room temperature. The fixing solution should be stored in a dark and ventilated place at 18℃-20℃.