未脱钙骨硬组织切片 Goldner 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
Goldner 染液套装	Servicebio	G1064
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：依次将切片入乙二醇乙醚乙酸酯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、细胞核染色：Goldner 染液 A 与 Goldner 染液 B 液等比混合，切片入混合液染色 20 min，自来水洗，1%盐酸酒精溶液分化 2 s，自来水冲洗，蒸馏水洗。

3、Goldner 染液 C：切片入第一缸 Goldner 染液 C 浸染 5-10 min，0.2%冰醋酸快速漂洗每缸 3 s。

4、Goldner染液 D染色：切片入Goldner染液 D浸染3 min，显微镜下控制，以胶原处Goldner染液C褪色为准。

5、Goldner染液 C染色：切片第二缸Goldner染液 C染3-5 min，0.2%冰醋酸快速漂洗每缸3

s。

6、Goldner染液E染色：切片入Goldner染液E染3-5 min，0.2%的冰醋酸三缸分化每缸2s，无水乙醇三缸脱水各2 s、3 s、5 s。

7、透明封片：切片放入第三缸无水乙醇5 min，二甲苯透明5 min，中性树胶封片。

8、显微镜镜检，图像采集分析。

三、 结果判读：

细胞核蓝紫色，矿化骨绿色，非矿化骨是橙红色。

四、 注意事项：

1、根据切片的数量对铁苏木素染液定期进行更换，也可以现配现用。

2、Goldner 染液 D 与 Goldner 染液 C 的染色程度要控制好，胶原部分不能是太红，会影响 Goldner 染液 D 的着色。

3、冰醋酸分化 Goldner 染液 E 的过程中，要分化适当，不能过深或者过浅。

Undecalcified Bone Goldner Stain Report

1. Experimental equipment and reagent

1.1 Equipment

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

1.2 Reagents

Reagent	Manufactures	Cat
Ethanol absolute	Sinopharm Chemical Reagent Co.,Ltd	100092683
Xylenes	Sinopharm Chemical Reagent Co.,Ltd	100023418
Goldner staining solution	Servicebio	G1064
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 Nuclear stain

Goldner staining solution A was mixed with Goldner staining solution B in an equal ratio, and the sections were put into the mixed solution for 20 minutes. Rinse with running water. Differentiated with 1% hydrochloric acid-alcohol solution for 2 s, washed with running water and rinse with

distilled water.

2.3 Goldner staining solution C

Put sections into the first Goldner staining solution C for 5-10 minutes and then rinse with 0.2% glacial acetic acid for 3 s.

2.4 Goldner staining solution D

The sections were soaked in Goldner staining solution D for 3 minutes and observe under the microscope. The criterion is the red color of the collagen fades.

2.5 Goldner staining solution C

The sections were dyed by another cylinder of Goldner dyeing liquid C for 3-5 min. Each cylinder was quickly rinsed with 0.2% glacial acetic acid for 3 s.

2.6 Goldner staining solution E

Put sections into Goldner staining solution E for 5-10 minutes and then rinse with 0.2% glacial acetic acid for 3 s. Finally, sections were dehydrated by ethyl alcohol for 2s, 3 s and 5 s.

2.7 Transparent and Mounting

Put the sections into three cylinders of anhydrous ethanol for 5 min and two cylinders of xylene for 5 minutes. Finally, sealed the sections with neutral balsam.

2.8 Microscope observation and collection and analysis images.

3. Result determination

The nucleus is bluish purple, the mineralized bone is green, and the non-mineralized bone is salmon red.

4. Matter need attention

4.1 The degree of staining of Goldner staining solution D and Goldner staining solution C should be controlled. Collagen should not be too red or will affect the staining of Goldner staining solution D.

4.2 During the process of glacial acetic acid differentiation of Goldner staining solution E, the differentiation should be appropriate, not too dark or too light.