# 骨组织脱钙实验报告（EDTA 慢脱）

## 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 |
| 组织包埋框 | 福建启盛实验设备科技有限公司 | 58444 |
| 石蜡 | 广东大川特种蜡有限公司 | |
| 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 |
| 搅拌机 | 大龙兴创实验仪器（北京）股份公司 | OS20-Pro |
| 分析天平 | 上海瑶新电子科技有限公司 | LQ-A20002 |

### 2、主要试剂及货号

| 名称 | 厂家 | 型号 |
|---|---|---|
| 乙二胺四乙酸二钠 | 国药集团化学试剂有限公司 | 10009717 |
| 氢氧化钠 | 国药集团化学试剂有限公司 | 10019718 |

## 二、石蜡切片制作

**1、取材：**将新鲜组织用固定液固定 24h 以上。固定完成后，将固定液从装有组织的 EP 管中倒出，然后向 EP 管内倒满配置好的脱钙液，密封，进行脱钙。

**2、脱钙：**将脱钙的组织贴好标示，温度一般控制在 25℃至 30℃之间，脱钙液每 3 天更换一次，用小针头刺骨组织（非目的区），可轻松扎透说明脱钙已彻底。如遇特大组织，需边脱钙边用切片刀按取材要求剖开，使组织脱钙彻底。

**3、脱水浸蜡：**将软化好的骨组织取出，放入对应编号的包埋框内，用流水冲洗后，放入脱水机内依次梯度酒精进行脱水与浸蜡。

**4、包埋：**将浸好蜡的组织于包埋机内进行包埋。先将融化的蜡放入包埋底模内，待底模内的蜡稍凝固，将组织从包埋框内取出，按照包埋面的要求放入包埋底模，用镊子底部轻压组织，使组织完全平整的在底模内。将对应编号的包埋框盖在装有组织的底模上，轻轻移至-20℃冻台冷却，待蜡凝固后，将蜡块从底模中取出并修整蜡块。

**5、切片：**将修整好的蜡块置于石蜡切片机切片，厚 4μm。切片漂浮于摊片机 42℃温水上将组织展平，用载玻片将组织垂直捞起，待组织上的水稍沥干后，放入 60℃烤片机内烤片，烤片时间为 30min 至 2h，待水烤干蜡烤化后，取出常温保存备用。

## 三、注意事项

不可用针刺目的区，针头要小，如骨组织有皮肤、肌肉粘附，可在脱钙前进行剥离。

# Experimental Report on Decalcification of Bone Tissue (EDTA)

## 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 |
| Embedding frame | Fujian Qisheng Experimental Equipment Technology Co., Ltd | 58444 |
| Microtome | ThermoFisher Scientific | SHANDON FINESSE 325 |
| Paraffin | Guangdong Dachuan Special Wax Co., Ltd | |
| 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 |
| Mixer | DLAB Scientific Co., Ltd | OS20-Pro |
| Analytical balance | Shanghai Yaoxin Electronic Technology Co., Ltd | LQ-A20002 |

1.2 Key reagents

| Reagent | Manufacture | Specifications/Model |
|---|---|---|
| EDTA-2Na | Sinopharm | 10009717 |
| NaOH | Sinopharm | 10019718 |

## 2. Procedures

2.1 The fresh tissue was fixed with fix solution for more than 24 hours. The fix solution was poured out of the EP tube containing tissue after fixation. Then, the EP tube was filled with the prepared decalcifying solution and sealed.

2.2 Decalcification: The decalcified tissue shall be labeled and the temperature shall be generally controlled between 25℃ and 30℃. The decalcified solution shall be replaced every 3 days. A small needle can be used to stab bone tissue (not target area), which can be easily pierced, indicating that decalcification is complete.

2.3 Tissue processing: The decalcified bone tissue was taken out from the tube and put into the embedding frame with corresponding label. Tissues were washed with running water and then put into tissue processor for dehydration and wax immersion.

2.4 Paraffin embedding: The wax-soaked tissue was embedded by using tissue embedder. Firstly, melted paraffin was put in the bottom of the embedded mold. Then, tissue was taken out of the embedding frame when the paraffin solidified slightly and put into embedded mold according to the requirements of the different section. Tweezers were used to gently press the tissue to make the tissue completely flat at the bottom of mold. Finally, the mold with tissue in it was covered by embedding frame with corresponding identifier and moved to the -20℃ freezing table for cooling. The paraffin block was taken out from the mold after paraffin solidified.

2.5 The trimmed paraffin block was put on the microtome to be sliced with 4μm thickness. Sections were floated on warm water (42℃) to be flatten and were picked up vertically by using a glass slide. After tissue was dried, sections were put into incubator at 60℃ for 30 minutes to 1 hour. Sections were taken out of the incubator and stored at room temperature.

## 3. Attention

The target area cannot be punctured. The needle head should be small. If the bone tissue has skin and muscle adhesion, it can be stripped before decalcification.

**4. Technical support**

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

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