

骨组织脱钙石蜡包埋实验报告

一、实验器材及试剂

1、实验器材

| 名称 | 厂家 | 型号 |
|------|------------|-------------|
| 脱水机 | 武汉俊杰电子有限公司 | JJ-12J |
| 包埋机 | 武汉俊杰电子有限公司 | JB-P5 |
| 冻台 | 武汉俊杰电子有限公司 | JB-L5 |
| 微波炉 | 格兰仕 | P70D20TL-D4 |
| 恒温摇床 | BLABOTERY | ZHPW-250 |
| 电子秤 | ELECTRONIC | SF-400 |

2、主要实验试剂

| 试剂 | 厂家 | 货号 |
|----------|--------------|-----------|
| 二甲苯 | 国药集团化学试剂有限公司 | 10023418 |
| 无水乙醇 | 国药集团化学试剂有限公司 | 100092683 |
| EDTA 脱钙液 | Wanwu | G1105 |

二、脱钙流程

- 取材：**将打印好标签与组织一一对应放在有孔的 PE 管或者是包埋框中，然后放入桶中，倒满新型脱钙液，密封，放入恒温摇床中，进行恒温摇床脱钙。脱钙液更换周期为 2-3d。
- 脱钙：**将脱钙小桶放入恒温摇床内进行脱钙处理，温度一般控制在 25 摄氏度到 30 摄氏度之间。恒温摇床的速率控制在 110-120RPM，每两天观察一次脱钙程度，若用针可扎的动时，用莱卡刀片将组织按照取材要求剖开，可加速软化速度。
- 脱水浸蜡：**将软化好的骨头取出，用流水冲洗一下午，放入脱水吊篮，于脱水机内依次梯度酒精进行脱水。75%酒精 2h-85%酒精 2h-90%酒精 1.5h-95%酒精 2h-无水乙醇 I 2h-无水乙醇 II 2h-醇苯 40min-二甲苯 I 40min-二甲苯 II 40min-65℃融化石蜡 I 0.5h-65℃融化石蜡 II 1h-65℃融化石蜡 III 2h, 45min。
- 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框，待蜡凝固之前将组织从脱水盒内取出按照包埋面的要求放入包埋框并贴上对应的标签。于-20℃冻台冷却，蜡凝固后将蜡块从包埋框中取出并修整蜡块。
- 切片：**将修整好的蜡块置于石蜡切片机切片，厚 4 μ m。切片漂浮于摊片机 40℃ 温水上将组织展平，载玻片将组织捞起，60℃ 烘箱内烤片。水烤干蜡烤化后取出常温保存备用。

Experimental report on decalcification and paraffin embedding of bone tissue

1. Experimental equipments and reagents

1.1 Experimental equipments

| Name | Factory | Model |
|-----------------------------|------------------------------------|-------------|
| Dehydrator | Wuhan Junjie Electronics Co., Ltd. | JJ-12J |
| Embedding machine | Wuhan Junjie Electronics Co., Ltd. | JB-P5 |
| Frozen platform | Wuhan Junjie Electronics Co., Ltd. | JB-L5 |
| Microwave oven | Galanz | P70D20TL-D4 |
| Constant temperature shaker | BLabotery | ZHPW-250 |
| Electronic scale | Electronic | SF-400 |

1.2 Main experimental reagents

| Reagent | factory | Article number |
|----------------------------|---|----------------|
| Xylene | Sinopharm GroupChemical Reagent Co., Ltd. | 10023418 |
| Absolute ethanol | Sinopharm GroupChemical Reagent Co., Ltd. | 100092683 |
| EDTA decalcification fluid | Wanwu | G1105 |

2. Experimental steps

2.1 Material: place the printed labels and tissues one by one in a perforated PE tube or embedding frame, then put them in a bucket, pour the new decalcification solution, seal them, and put them in a constant temperature shaker In the process, decalcification is carried out by constant temperature shaker decalcification. The replacement period of the decalcification solution is 2-3d.

2.2 Decalcification: Put the decalcification keg into the constant temperature shaker for decalcification, and the temperature is generally controlled at 25 degrees Celsius Between 30 degrees Celsius. The rate of the constant temperature shaker is controlled at 110-120RPM, and the degree of decalcification is observed every two days.If the needle can be moved, the tissue is cut with a Lycra blade according to the material requirements, which can accelerate the softening speed.

2.3 Dehydrating wax: take out the softened bones, rinse with running water for an afternoon, put it into the dehydrating basket, and dehydrate in a gradient gradient alcohol in the dehydrator.Details as follow: 75% alcohol for 2h ; 85% alcohol for 2h; 90% alcohol for 1.5h; 95% alcohol for 2h;

anhydrous ethanol I for 2h; anhydrous ethanol II for 2h; alcohol benzene for 40min; Xylene I for 40min; Xylene II for 40min; 65°C melted paraffin I for 0.5h at 65°C; Melted paraffin II for 1h; 65°C Melted paraffin III for 2h45min.

2.4 Burying: The wax-soaked tissue is embedded in the embedding machine. Put the melted wax into the box first, remove the tissue from the box before the wax solidifies and put it into the box according to the accordingly. Cooling at -20°C freezing table, the wax block is removed from the embedding frame and trimmed after solidification.

2.5 Sectioning: Place the trimmed wax block in a paraffin section, 4μm. thick Slice float on the spreader 40°C warm water will spread the tissue flat, slide will remove the tissue, 60°C oven baking sheet. The water is roasted with dry wax and stored at room temperature.