

## 扫描电镜 EDS 实验报告

### 一、实验器材及试剂

#### 1、实验器材

名称	厂家	型号
临界点干燥仪	Quorum	K850
离子溅射仪	HITACHI	MC1000
扫描电子显微镜	HITACHI	SU8100
X 射线能谱仪 (EDS)	Oxford	ULTIMMAX 100

#### 2、主要实验试剂

试剂	厂家	货号
电镜固定液	wanwu	G1102
无水乙醇	国药集团化学试剂有限公司	100092183
乙酸异戊酯	国药集团化学试剂有限公司	10003128
PBS	wanwu	G0002
锇酸	Ted Pella Inc	

### 二、扫描电镜制样步骤

1、**取材固定**：新鲜组织确定取材部位，尽量减小牵拉、挫伤与挤压等机械损伤，1-3min 内取样，组织块面积不超过 3mm<sup>2</sup>，用 PBS 轻轻漂洗将样本表面的血污，毛发等去掉，保护好需要扫描的面并做好标记（如在对面进行剪角处理）。迅速投入电镜固定液室温固定 2h，再转移至 4°保存。

贴壁细胞：贴壁于盖玻片的细胞培养处理完成后弃培养基，用 PBS 轻轻漂洗后，弃 PBS 加电镜固定液室温固定 2h，再转移至 4°保存。注意保护好扫描面避免剧烈震荡细胞脱落。

2、**后固定**：固定好的样品经 0.1M 磷酸缓冲液 PB (PH7.4) 漂洗 3 次，每次 15min。0.1M 磷酸缓冲液 PB (PH7.4) 配制 1%锇酸室温避光固定 1-2h。0.1M 磷酸缓冲液 PB (PH7.4) 漂洗 3 次，每次 15min。

3、**脱水**：组织依次入 30%-50%-70%-80%-90%-95%-100%-100%酒精每次 15min,乙酸异戊酯 15min。

4、**干燥**：将样本放入临界点干燥仪内进行干燥。

（干燥的样品及无机材料不需要以上步骤）

5、**样本导电处理**：将样本紧贴于导电碳膜双面胶上放入离子溅射仪样品台上进行喷金 30s 左右。

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6、**元素分析**：扫描电子显微镜下观察待测区域元素分析。

点扫描：待测区域随机选取 3 个点，收集能量信号，检测每个点的元素含量，保存图谱及百分含量表。

线扫描：待测区域随机选定 1 条直线轨迹，并检测该直线上元素浓度的变化。曲线的高低起伏反映所对应元素沿着扫描线浓度的变化。

面扫描：样品表面某一区域作检测区域，通过采集特征 X 射线光子，可以得到每个元素的面分布图，每种元素由不同的颜色代表。

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## Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy Report

### 1. Apparatus and Reagents

#### 1.1 Major Apparatus

Name	Producer	Model
Critical Point Dryer	Quorum	K850
Lon Sputtering Apparatus	HITACHI	MC1000
Scanning Electron Microscope	HITACHI	SU8100
EDS	Oxford	ULTIMMAX 100

#### 1.2 Major Reagents

Name	Producer	Code
Fixative for TEM	wanwu	G1102
Ethanol	Sinaopharm Group Chemical Reagent Co. LTD	100092183
Isoamyl acetate	Sinaopharm Group Chemical Reagent Co. LTD	10003128
PBS	wanwu	G0002
OsO <sub>4</sub>	Ted Pella Inc	

### 2. Procedure

**2.1 Harvest tissue block and fixation:** Targeted fresh tissues should be selected to minimize mechanical damage such as pulling, contusion and extrusion. Use a sharp blade to cut and harvest fresh tissue blocks quickly within 1-3 minutes. The area of tissue block should be no more than 3 mm<sup>2</sup>. Wash tissues with PBS gently to remove the blood and hair, etc. Label the target side of tissue (the side you want to observe) by any way, such as making cuts on the opposite side. Make sure to protect tissue blocks, especially the target side, from mechanical damage such as forceps extrusion. The washed tissue blocks are immediately fixed by electron microscopy fixative for 2 hours at room temperature, then transferred into 4°C for preservation and transportation.

**For adherent cell:** Seed cells on a sterile cover glass in a petri dish. Remove the culture medium, then wash slide gently with PBS, followed by adding electron microscopy fixative into petri dish. After fixing for 2 hours at room temperature, transfer the petri dish to 4°C for preservation and transport. Note to avoid sever shock which may result in the cells dropping off from cover glass.

**2.2 Post-fix:** Wash tissue blocks with 0.1 M PB (pH 7.4) for 3 times, 15 min each. Then transfer tissue blocks into 1% OsO<sub>4</sub> in 0.1 M PB (pH 7.4) for 1-2 h at room temperature. After that, wash tissue blocks in 0.1M PB (pH 7.4) for 3 times, 15 min each.

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**2.3 Dehydrate as followed:**

30% ethanol for 15 min;  
50% ethanol for 15 min;  
70% ethanol for 15 min;  
80% ethanol for 15 min;  
90% ethanol for 15 min;  
95% ethanol for 15 min;  
Two changes of 100% ethanol for 15 min;  
Finally, isoamyl acetate for 15 min.

**2.4 Drying:** Dry samples with Critical Point Dryer.

**Note: Dry samples and inorganic materials should ignore all the steps above and do the following step directly.**

**2.5 Conductive metal coating:** Specimens are attached to metallic stubs using carbon stickers and sputter-coated with gold for 30s.

**2.6 Elemental analysis :**The element analysis of the area to be measured was observed under scanning electron microscope.

Point scanning: Three points were randomly selected from the area to be tested, energy signals were collected, element content of each point was detected, and atlas and percentage table were saved.

Line scan: A linear trajectory was randomly selected in the region to be tested, and the change of element concentration on the line was detected. The fluctuation of the curve reflects the change in the concentration of the corresponding element along the scan line.

Surface scan: A certain area on the surface of the sample is used as the detection area. By collecting characteristic X-ray photons, the plane distribution diagram of each element can be obtained, and each element is represented by different colors.