组织、细胞、细菌免疫电镜实验报告

一、 实验器材及试剂

1、实验器材

名称	厂家	型号
超薄切片机	Leica	Leica UC7
钻石切片刀	Daitome	Ultra 45°
透射电子显微镜	HITACHI	HT7800/HT7700
低温紫外聚合仪	中镜科仪	UVCC2515
高速冷冻离心机	THERMOFISHER	SORVALL ST 16R
藤原无油真空泵	Fujiwara	
150 目方华膜镍网	中镜科仪	BZ102615Na

2、主要实验试剂

试剂	厂家	货号
免疫电镜专用固定液	Wanwu	G1124-100ML
低熔点琼脂糖	Solarbio	A8350
品红	Wanwu	G1061-250ML
无水乙醇	国药集团化学试剂有限公司	100092183
LR White 树脂	海德生物	14381-UC
PBS	Wanwu	G0002
TBS	Wanwu	G0015-500ML
BSA	Wanwu	G5001
一抗		
二抗（10nm 胶体金 goat 抗 rab）	Sigma	G7402
二抗（10nm 胶体金 goat 抗 mouse）	Sigma	G7777

二、 实验步骤

1、取材固定

组织：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，取样组织 1mm^3 大小。取材前可提前准备装有免疫电镜固定液的培养皿，将小组织块离体取下后立即投入培养皿内，用手术刀在培养皿的固定液中进行切割成 1mm^3 的小组织块。再将切割好的小组织块转移至装有新的免疫电镜固定液的 EP 管内继续固定，4℃固定保存及运输。

细胞、细菌：离心收集细胞或细菌沉淀，要求沉淀最少绿豆大小。加入免疫电镜专用固定

液重悬混匀固定，4℃固定保存及运输。

2、**清洗**

组织：用 4℃预冷的 0.1M 磷酸缓冲液 PB（pH7.4）在冰盒上漂洗 3 次，每次 10min。

细胞、细菌：细胞或细菌用高速冷冻离心机 4℃离心，弃上清加入 4℃预冷的 0.1M 磷酸缓冲液 PB（PH7.4），混匀漂洗 3min 后再离心，重复洗涤 3 次。提前加热溶解制备 2%琼脂糖溶液，待冷却至 40℃左右后加入 EP 管内，在琼脂糖凝固之前将沉淀用镊子挑起悬浮包裹于琼脂糖内。在第一次漂洗时，可加入微量品红染液，使细胞着色，有利于后续切片定位。

3、脱水：预冷后的梯度酒精进行脱水，依次为 30%酒精 4℃ 20min，50%酒精-20℃ 20min，70%酒精-20℃ 20min，80%酒精-20℃10min，85%酒精-20℃ 10min，90%酒精-20℃ 10min，95%酒精-20℃ 10min，100%酒精-20℃ 10min，100%酒精-20℃ 10min。

4、树脂渗透：100%酒精：纯树脂=2:1 4℃ 1h；100%酒精：纯树脂=1:1 4℃ 3h；100%酒精：纯树脂=1:2 4℃ 17-20h；纯树脂 4℃ 17-20h；纯树脂 4℃ 2 次，1h/次。

5、包埋：4℃，先将纯树脂滴入包埋胶囊中，然后将样本放入纯树脂中并扣上胶囊帽进行包埋，抽真空 0.5h-1h 后扣紧胶囊帽。

6、聚合：低温紫外聚合仪-20℃进行聚合 48h 以上，后恢复室温，取出树脂块备用。

7、超薄切片：超薄切片机切片 70-80nm 超薄切片，镀膜镍网捞片，片子 4℃保存。

8、免疫标记：

①复温水合：镍网从 4℃取出用超纯水悬浮 5min；

②清洗：TBS 室温清洗 3 次，每次 5min；

③封闭：1%BSA/TBS 的封闭液室温封闭 30min；

④加一抗：抗体与封闭液按一定稀释比稀释后 4℃过夜孵育；

⑤清洗：室温复温后，TBS 清洗 3 次，每次 5min；

⑥加二抗：二抗与二抗稀释液按一定稀释比稀释，先室温孵育 20min，后转 37℃烘箱孵育 1h，然后转室温复温 0.5h。实验过程中防止干片。

⑦清洗：TBS 清洗 5 次，每次 5min 后，超纯水清洗 5 次，每次 5min。

⑧铀复染：镍网于 2%醋酸铀饱和酒精溶液避光染色 8min；70%酒精清洗 3 次；超纯水清洗 3 次。

⑨烘干：清洗后的镍网用滤纸吸干后放入网板中，37℃烘箱放置 10min 烘干备用。

9、结果观察图像采集：在透射电子显微镜下观察并采集图像，黑色 10nm 大小的金颗粒即为阳性表达。

Immune Electron Microscopy(IEM) Report for tissues, Cells and Bacteria

1. Apparatus and Reagents

1.1 Major Apparatus

Name	Producer	Model
Ultra Microtome	Leica	Leica UC7
Diamond slicer	Daitome	Ultra 45°
Transmission electron microscope	HITACHI	HT7800/HT7700
Low temperature UV polymerizer	Electron Microscopy China	UVCC2515
High speed freezing centrifuge	THERMOFISHER	SORVALL ST 16R
Fujiwara oil-free vacuum pump	Fujiwara	
150 meshes nickel grids with formvar film	Beijing Zhongjing Keyi	BZ102615Na

1.2 Major Reagents

Name	Producer	Code
Fixative for IEM	Wanwu	G1124-100ML
Low gelling temperature agarose	Solarbio	A8350
Azaleine	Wanwu	G1061-250ML
Ethanol	Sinaopharm Group Chemical Reagent Co. LTD	100092183
LR White Resin	HaideBio	14381-UC
PBS	Wanwu	G0002
TBS	Wanwu	G0015-500ML
BSA	Wanwu	G5001
Primary antibody		
10nm labeled goat anti-rabbit secondary antibody	Sigma	G7402
10nm labeled goat anti-mouse secondary antibody	Sigma	G7777

2. Procedure

2.1 Harvest tissue block and fixation:

For tissues: 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 size of tissue block should be no more than 1 mm^3. Before sampling, petri dishes with fixative for IEM should be prepared in advance, small tissue blocks could be removed from animal body and immediately put into petri dishes, and then cut into small size of 1 mm^3 in the fixative. The 1mm^3 tissue blocks were transferred into an EP tube with fresh IEM fixative for further fixation, which was fixed at 4℃ for preservation and transportation.

For cells and bacteria: Collect cells or bacteria precipitation after centrifuge, requiring the precipitation should be at least mung beans size. The IEM fixative was added to the tube and let the precipitation re-suspended in the fixative, and then fixed at 4 ℃ for preservation and transportation.

2.2 Cleaning

For tissues: Wash tissue blocks on the ice box with pre-cooled 0.1 M PB (pH 7.4) for 3 times, 10 min each.

For cells and bacteria: The fixed cells and bacteria were centrifuged at 4℃ in a high-speed refrigerated centrifuge. The pre-cooled 0.1 M PB (pH 7.4) at 4℃ was added into the tube after supernatant was discarded, and then the precipitation was re-suspended and washed in PB for 3min. This washing step was repeated for 3 times. The 2% low melting point agarose solution was prepared by heating and dissolving in advance. After being cooled to about 40℃, the agarose solution was added into the EP tube. Before agarose solidification, the precipitation was suspended with forceps and wrapped in the agarose. At the first rinse, a little of azaleine can be added to the precipitation to stain the cells or bacteria, which facilitate the positioning of subsequent sections.

2.3 Dehydrate as followed:

30% ethanol for 20 min at 4℃ ;
50% ethanol for 20 min at -20℃;
70% ethanol for 20 min at -20℃;
80% ethanol for 10 min at -20℃;
85% ethanol for 10 min at -20℃;
90% ethanol for 10 min at -20℃;
95% ethanol for 10 min at -20℃;
100% ethanol for 10 min at -20℃;
100% ethanol for 10 min at -20℃;

2.4 Resin penetration as followed:

100% ethanol: LR white resin=2:1 for 1h at 4℃ ;
100% ethanol: LR white resin=1:1 for 3h at 4℃ ;
100% ethanol: LR white resin=1:2 for 17-20h at 4℃ ;
Pure LR white resin for 17-20h at 4℃;

Two changes of pure LR white resin for 1 h at 4℃;

2.5 Embedding: At 4℃, the pure LR white resin was first dropped into the embedding capsule, then the sample was put into the pure resin and the capsule cap was covered loosely at first. After vacuum extraction for 0.5-1h, the capsule cap was fastened for embedding.

2.6 Polymerization: The capsules with resin and samples were moved into low temperature UV polymerizer to polymerize for more than 48h at -20℃. And then the resin blocks were taken out from the capsules for standby application at room temperature.

2.7 Ultrathin section: The resin blocks were cut to 70-80nm thin on the ultra microtome, and the tissues were fished out onto the 150 meshes nickel grids with formvar film. The nickel grids with tissues were stored at 4℃ for standby application.

2.8 Immunolabelling

2.8.1 The nickel grids with tissues were taken out from the 4℃ fridge and then suspended on the ultra pure water for 5min.

2.8.2 Rinsed the nickel grids on TBS drop for 3 times, 5min each at room temperature.

2.8.3 Blocked the tissues in 1%BSA TBS solution for 30min at room temperature.

2.8.4 The nickel grids were incubated in the primary antibody diluted in 1%BSA TBS solution at some ratio overnight at 4℃.

2.8.5 The second day, the nickel grids were taken out and recovered to room temperature, and then rinsed the nickel grids on TBS drop for 3 times, 5min each at room temperature.

2.8.6 The nickel grids were incubated in the the secondary antibody diluted in diluent for 20min at room temperature, and then incubated for 1h at 37℃, at last incubated for 0.5h at room temperature. Make sure the nickel grids kept wet.

2.8.7 Rinsed the nickel grids on TBS drop for 5 times, 5min each at room temperature. And then rinsed the nickel grids on ultra pure water drop for 5 times, 5min each at room temperature.

2.8.8 2% uranium acetate saturated alcohol solution avoid light staining for 8 min, rinsed in 70% ethanol for 3 times and then rinsed in ultra pure water for 3 times.

2.8.9 After washed, the nickle grids were dried on filter paper and then put into grids board. The nickle grids were dried in 37℃ oven for 10min for standby application.

2.9 Observation and images capture: The nickle grids are observed under TEM and take images. The 10nm black golden particles are positive signals.