冰冻切片免疫组化实验报告

一、实验原理

免疫组化，是应用免疫学基本原理——抗原抗体反应，即抗原与抗体特异性结合的原理，通过化学反应使标记抗体的显色剂（荧光素、酶、金属离子、同位素）显色来确定组织细胞内抗原（多肽和蛋白质），对其进行定位、定性及定量的研究，称为免疫组织化学技术（IHC）或免疫细胞化学技术（ICC）。

二、实验器材及试剂

1、实验器材

名称	厂家	型号
冰冻切片机	达科为深圳医疗设备有限公司	6250
烤箱	天津市莱玻特瑞仪器设备有限公司	GFL125
微波炉	美的	M1-L213B
盖玻片	江苏汇达医疗器械有限公司	710510
载玻片	海门市神鹰实验仪器厂	188109
脱色摇床	武汉赛维尔生物科技有限公司	SYC-Z100
涡旋仪	武汉赛维尔生物科技有限公司	MX-F
掌上离心机	武汉赛维尔生物科技有限公司	DS-S 100
显微镜	NIKON	ECLIPSE E100
组化笔	Gene tech	GT1001
移液枪	Dragon	KE003068
江丰扫描仪	宁波江丰生物信息技术有限公司	KF-PRO-120

2、主要实验试剂

试剂	厂家	货号
OCT 包埋剂	杭州浩克生物技术有限公司	HK2061
纯甲醇	上海凌峰化学试剂有限公司	67561
无水乙醇	杭州宏达化工仪器有限公司	SJ003614
二甲苯	国药集团化学试剂有限公司	10023418
抗原修复液 EDTA(9.0)	杭州浩克生物技术有限公司	HKI0004
PBS 缓冲液	杭州浩克生物技术有限公司	HK0002
BSA 牛血清白蛋白	杭州浩克生物技术有限公司	HKW2084
苏木素染液	杭州浩克生物技术有限公司	HK2053
苏木素分化液	杭州浩克生物技术有限公司	HK2054
苏木素返蓝液	杭州浩克生物技术有限公司	HK2055
中性树胶	国药集团化学剂有限公司	10004160
一抗：		
二抗：HRP 超敏山羊抗兔鼠通用二抗	杭州浩克生物技术有限公司	HKI0029
DAB 显色剂	Proteintech	PR30010

三、实验步骤

1. **冰冻切片固定：**冰冻切片室温晾干复温，置于纯甲醇固定 10min，后于 37℃或室温干燥，在 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。
2. **抗原修复：**组织切片置于盛满 EDTA 抗原修复缓冲液（PH9.0）的修复盒中于微波炉内进行抗原修复。中低火 10min，此过程中应防止缓冲液过度蒸发，切勿干片。自然冷却后将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。（修复液和修复条件根据组织来确定）

3. **阻断内源性过氧化物酶：**切片放入 3%过氧化氢溶液，室温避光孵育 25min，将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。
4. **画圈：**用组化专用的组化笔沿着组织外围轮廓画一个与组织间隔 3-4 毫米的小圈，然后加入足量的 PBS 保证后续依次加入的封闭血清，一抗，二抗，以及显色剂能完全覆盖组织，而不沿着玻片流走。
5. **血清封闭:**切片稍甩干后用组化笔在组织周围画圈（防止抗体流走），在圈内滴加用 3%BSA 室温封闭 30min 以上。
6. **加一抗：**轻轻甩掉封闭液，在切片上滴加 PBS 按一定比例配好的一抗，切片平放于湿盒内 4°C 孵育过夜。（湿盒内加少量水防止抗体蒸发）
7. **加二抗：**玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。切片稍甩干后在圈内滴加与一抗相应种属的二抗（HRP 标记）覆盖组织，室温孵育 50min。
8. **DAB 显色：**玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。切片稍甩干后在圈内滴加（稀释液和浓缩液 1000:50 配制）新鲜配制的 DAB 显色液，显微镜下控制显色时间，阳性为棕黄色，纯水冲洗切片终止显色。
9. **复染细胞核：**苏木素染色 2-3mins 左右，自来水洗，苏木素分化液分化 2 秒，自来水冲洗，苏木素返蓝液返蓝 15-30s，流水冲洗。
10. **脱水封片：**将切片依次放入 75%酒精 4min—85%酒精 4min—95%酒精 4min—无水乙醇 4min—二甲苯I 4min—二甲苯II 4min 中脱水透明，将切片从二甲苯拿出来稍晾干，中性树胶封片。

四、结果判读

苏木素染细胞核为蓝色，DAB 显出的阳性表达为棕黄色。

五、注意事项

1. 实验过程中切片勿干片；
2. 实验操作中小心枪头挂伤组织；
3. 冰冻切片注意防脱。

Frozen section immunohistochemical experiment report

1. Experimental principle

Immunohistochemistry is the application of the basic principle of immunology - antigen-antibody reaction, that is, the principle of specific combination of antigen and antibody, through chemical reactions to label antibody chromogenic agents (fluorescein, enzymes, metal ions, isotopes) to determine the antigen (polypeptide and protein) in tissue cells, and to locate, qualitative and quantitative research on it. These are called immunohistochemistry (IHC) or immunocytochemistry (ICC).

2. Laboratory equipment and reagents

2.1 Laboratory equipment

Equipment	Manufacturers	Model
Frozen microtome	DAKEWE	6250
Oven	Tianjin Leibo Terry Equipment Co., Ltd	GFL125
Microwave oven	Midea	M1-L213B
Coverslips	Jiangsu Huida Medical Instruments Co., Ltd	710510
Glass microscope slides	Haimen Shenying Experimental Equipment Factory	188109
Rocker	Servicebio	SYC-Z100
Vortex	Servicebio	MX-F
Micro-centrifuge	Servicebio	DS-S 100

Microscope	NIKON	ECLIPSE E100
Liquid Blocker PAP Pen	Gene tech	GT1001
Pipettor	Dragon	KE003068
Imaging System	Konfoong Bioinformation Tech Co.,Ltd	KF-PRO-120

2.2 Laboratory reagents

Reagents	**Manufacturers**	**Catlog**
OCT embedding agent	Hangzhou Haoke Biotechnology Co., Ltd	HK2061
Methyl alcohol	Shanghai Lingfeng Chemical Reagent Co., Ltd	67561
Anhydrous ethanol	Hangzhou Hongda Chemical Instrument Co., Ltd	SJ003614
Xylene	Sinopharm Chemical Reagent co., Ltd.	10023418
EDTA Antigen repair solution (9.0)	Hangzhou Haoke Biotechnology Co., Ltd	HKI0004
PBS solution	Hangzhou Haoke Biotechnology Co., Ltd	HK0002
BSA	Hangzhou Haoke Biotechnology Co., Ltd	HKW2084
Hematoxylin staining solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2053
Hematoxylin differentiate solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2054

Hematoxylin bluing solution	Hangzhou Haoke Biotechnology Co., Ltd	HK2055
Neutral gum	Sinopharm Chemical Reagent co., Ltd.	10004160
Primary antibody： Second antibody：HRP hypersensitive goat anti-rabbit secondary antibody	Hangzhou Haoke Biotechnology Co., Ltd	HKI0029
DAB dyeing solution	Proteintech	PR30010

3. Experimental procedure

3.1 Frozen section fixation: The frozen slices were taken out of the refrigerator and rewarmed, dried, fixed in pure methanol for 10min, then dried at 37 ℃ or room temperature, and washed three times on a decolorizing shaker in PBS (PH7.4) for 5min each time.

3.2 Repair: The tissue sections were placed in a repair box filled with EDTA antigen repair buffer (PH9.0) for antigen repair in a microwave oven. Medium-low fire for 10min, during this process should prevent excessive evaporation of buffer, do not dry. After natural cooling, the slide was placed in PBS (PH7.4) and washed by shaking on the decolorizing shaker for 3 times, 5min each time. (Repair solution and repair strength are determined according to the tissue)

3.3 Block endogenous peroxidase: The sections were incubated with 3% hydrogen peroxide at room temperature for 25min. The slides were placed in PBS (PH7.4) and washed three times on a decolorizing shaker for 5min each time.

3.4 Draw a circle around the tissue: Use a histochemical pen to draw a small circle 3-4 mm apart from the tissue along the outer outline of the tissue, and then add enough PBS to

ensure that the subsequent addition of blocking serum, primary antibody, secondary antibody, and color development agent can completely cover the tissue without flowing along the sections.

3.5 Serum blocking: After the section is slightly dried, draw a circle around the tissue with a histochemical pen (to prevent the antibody from flowing away), and add 3%BSA to the circle and seal it at room temperature for more than 30 minutes.

3.6 Add primary antibody: Gently shake off the sealing solution, add PBS to the slices with a certain proportion of primary antibody, and the slices are placed flat in a wet box at 4° C for overnight incubation. (Add a small amount of water to the wet box to prevent the antibody from evaporating)

3.7 Add secondary antibody: The sections were placed in PBS (PH7.4) and washed by shaking on the decolorizing shaker for 3 times, 5min each time. After the slices were slightly dried, the tissue covered by the second antibody of the corresponding species of the first antibody was added to the ring and incubated at room temperature for 50min away from light.

3.8 DAB color development: The slides were placed in PBS (PH7.4) and washed by shaking on the decolorizing shaker for 3 times, 5min each time. After the sections were slightly dried, the freshly prepared DAB color developing solution (prepared by diluent and concentrated solution 1000:50) was added into the circle. The color developing time was controlled under the microscope. The positive color was brown and yellow, and the section was washed with pure water to terminate the color development.

3.9 Restaining of nucleus: Hematoxylin restained the nuclei for 2-3min. Wash with tap water, hematoxylin differentiate solution differentiation for 2s, rinse with tap water, hematoxylin bluing solution back blue 15-30s, water rinse.

3.10 Dehydration and sealing: Put the sections into 75% Ethyl alcohol for 4 min - 85% Ethyl alcohol for 4 min - 95% Ethyl alcohol for 4 min - Anhydrous ethanol for 4 min-

xylene I for 4 min - xylene II for 4 min, after slightly drying, , take the slices out of xylene for a little dry, and seal them with neutral gum.

4. Interpretation of results

The nucleus of hematoxylin stain is blue, and the positive signal of DAB is brown-yellow.

5. Precautions

5.1 Don't let the slices dry out during the experiment；

5.2 Be careful of the injured tissue on the pipette suction head during experimental operation；

5.3 Pay attention to prevent peeling of frozen slices.