# 细胞爬片免疫组化实验报告

## 一、实验器材及试剂

### 1、 实验器材

| 名称 | 厂家 | 型号 |
|---|---|---|
| 载玻片 | Wanwu | |
| 涡旋混合器 | Wanwu | MX-F |
| 掌上离心机 | Wanwu | D1008E |
| 脱色摇床 | Wanwu | TSY-B |
| 移液枪 | Dragon | KE0003087/KA0056573 |
| 显微镜 | Nikon | E100 |
| 组化笔 | Gene tech | GT1001 |

### 2、 主要实验试剂

| 试剂 | 厂家 | 货号 | 稀释比 |
|---|---|---|---|
| 无水乙醇 | 国药集团化学试剂有限公司 | 100092683 | |
| PBS 缓冲液 | Wanwu | G0002 | |
| 破膜工作液 | Wanwu | G1204 | |
| 正常兔血清 | Wanwu | G1209 | |
| BSA | Wanwu | G5001 | |
| 苏木素染液 | Wanwu | G1004 | |
| 苏木素分化液 | Wanwu | G1039 | |
| 苏木素返蓝液 | Wanwu | G1040 | |
| 中性树胶 | Wanwu | G1403 | |
| 一抗： | | | |
| 二抗： | | | |
| 组化试剂盒 DAB 显色剂 | Wanwu | G1211 | |

## 二、 免疫细胞化学实验步骤

1、细胞破膜：爬片稍甩干后用组化笔在盖玻片中间细胞分布均匀的位置画圈（防止抗体流走），加 50-100μL 破膜工作液，室温孵育 20min，PBS 洗 3 次，每次 5 min。

2、血清封闭:在组化圈内滴加 3%BSA 均匀覆盖组织，室温封闭 30min。（一抗是山羊来源

的用兔血清封闭，其他来源的用 BSA 封闭）。

3、加一抗：轻轻甩掉封闭液，在细胞孔板里滴加 PBS 按一定比例配好的一抗，1.细胞培养板平放于湿盒内 4°C 孵育过夜。

4、加二抗：细胞孔板置于脱色摇床上晃动洗涤 3 次，每次 5min。稍甩干后在圈内滴加组化试剂盒内与一抗相应种属的二抗（HRP 标记）覆盖组织，室温孵育 50min。

5、DAB 显色：细胞孔板置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。稍甩干后在圈内滴加新鲜配制的 DAB 显色液，显微镜下控制显色时间，阳性为棕黄色，自来水冲洗切片终止显色。

6、复染细胞核：滴加苏木素复染 3min 左右，水洗，滴加苏木素分化液分化数秒，水洗，滴加苏木素返蓝液返蓝，水洗。

7、脱水封片：往细胞孔板依次加入 75%酒精 5min--85%酒精 5min --无水乙醇Ⅰ5min --无水乙醇Ⅱ5min 中脱水，将盖玻片从酒精中拿出来吹风机吹干后将有细胞的一面朝下用中性树胶封固在载玻片上。

8、显微镜镜检，图像采集分析。

三、 免疫细胞化学结果判读

苏木素染细胞核为蓝色，DAB 显出来的阳性细胞为棕黄色。

# Immunohistochemical Experiment Report of Cell Crawling

I Experimental equipments and reagents

1.Experimental equipments

| Name | Manufacturer | Model |
|---|---|---|
| Slide | Wanwu | |
| Vortex mixer | Wanwu | MX-F |
| Palm centrifuge | Wanwu | D1008E |
| Decolorization shaker | Wanwu | TSY-B |
| Pipette gun | Dragon | KE0003087/KA0056573 |
| Microscope | Nikon | E100 |
| Tissue pencil | Gene tech | GT1001 |

2.Main experimental reagents

| Reagent | Manufacturer | Model | Dilution Ratio |
|---|---|---|---|
| Anhydrous ethanol | China National Pharmaceutical Group Chemical Reagent Co., LTD | 100092683 | |
| Phosphate buffer saline | Wanwu | G0002 | |
| membrane rupture working solution | Wanwu | G1204 | |
| Normal rabbit serum | Wanwu | G1209 | |
| Bovine serum albumin | Wanwu | G5001 | |
| Hematoxylin stain solution | Wanwu | G1004 | |
| Hematoxylin differentiation solution | Wanwu | G1039 | |
| Hematoxylin returning blue solution | Wanwu | G1040 | |
| Neutral gum | Wanwu | G1403 | |
| Primary antibody: | | | |
| Secondary antibody: | | | |

II Experimental procedure of immunocytochemistry

1. 1. Cell membrane rupture:after the slide was slightly shaken dry, a tissue pencil was used to draw a circle in the middle of the cover glass where the cells are evenly distributed (to prevent the antibody from flowing away). add 50-100μL of membrane rupture working solution, incubate at room temperature for 20 minutes, and wash with PBS(PH7.4) 3 times, 5 minutes each time.
2. Serum sealing: 3%BSA was added to the circle to evenly cover the tissue, and the tissues are sealed for 30 minutes at room temperature. (Primary antibody is sealed with normal rabbit serum from goat source and other sources are sealed with BSA).
3. Primary antibody incubation: the sealing solution is gently removed, the primary antibody prepared with PBS(PH7.4) in a certain proportion is added to the sections, and the sections are placed flat in a wet box and incubated overnight at 4℃.
4. Secondary antibody incubation: the sections are placed in PBS(PH7.4) and washed by shaking on the decolorizing shaker 3 times for 5 minutes each. After the sections are slightly shaken and dried, the tissues are covered with secondary antibody from the corresponding species of primary antibody and incubated at room temperature for 50 minutes.
5. DAB chromogenic reaction: the sections are placed in PBS(PH7.4) and shaken on the decoloring shaker 3 times for 5 minutes each. DAB color developing solution newly prepared is added in the circle after the sections are slightly dried. The color developing time is controlled under the microscope. The positive is brownish yellow.Rinse the sections with tap water to stop the reaction.
6. Nucleus counterstaining: the sections are counterstained with drop hematoxylin stain solution for about 3 minutes; washed with pure water; differentiated with drop hematoxylin differentiation solution for several seconds; washed with pure water; treated with drop hematoxylin returning blue solution; washed with running water.
7. Dehydration and mounting: place the section in 75% alcohol for 5 minutes--85% alcohol for 5 minutes--absolute ethanol I for 5 minutes--anhydrous ethanol II for 5 minutes, dehydrated and transparent, remove the sections from anhydrous ethanol and let them dry slightly with a hair dryer, then mount the sections with neutral gum.
8. Visualize staining of tissue under a microscope, acquisitive and analysis image.

III Interpretation of the immunocytochemistry results

The nucleus of hematoxylin stained is blue, and the positive expression of DAB is brownish yellow.