细胞滴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	载玻片
盖玻片	江苏世泰实验器材有限公司	10212432C
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
组化笔	Wanwu	WG1066-1
显微镜	Nikon	E100
成像系统	日本尼康	Nikon DS-U3

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	
二甲苯	上海凌峰化学试剂有限公司	1330-20-7	
PBS 缓冲液	Wanwu	G0002	
BSA	Wanwu	G5001	
破膜液	Wanwu	G1204	
4%多聚甲醛	Wanwu	G1101	
中性树胶	国药集团化学试剂有限公司	10004160	
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、 取细胞悬液，2800rpm 4℃离心 5min，弃上清液，根据底部沉淀的细胞量加入 2mL 多聚甲醛固定。若肉眼看不见细胞沉淀时，用 3000rpm，4℃离心 10min。

2、取用多聚甲醛重悬的细胞悬液，2800rpm 25℃离心 5min，弃上清液，根据底部沉淀加入PBS：①若肉眼看不见细胞沉淀时，用 3000rpm，25℃离心 10min，依然无沉淀时，留取底部约 0.2mL 的液体，再加入 0.5mL 的 PBS 混匀后用移液枪吸取 200μL，滴于提前用组化笔画好的小圆圈中（3cm×2cm 的椭圆）；②若细胞沉淀量很少，绿豆大小时，弃上清，留取底部沉淀，加入 0.5mL 的 PBS,用移液枪吹打混匀后，吸取 200μL，涂于提前用组化笔画好的小圆圈中（3cm×2cm 的椭圆)；③若细胞沉淀量很多，黄豆大小或更大时，弃上清，留取底部沉淀，加入 2mL PBS,用移液枪吹打混匀后，吸取 150μL，涂于提前用组化笔画好的大圆圈中（最大长边约 5cm，最大短边约 2cm 的椭圆），在显微镜下观察细胞量的多少，若还是过厚，再用 PBS 对倍稀释该细胞悬液，直至在显微镜下观察到细胞量涂布均匀。

3、用枪将细胞悬液铺满整个圆圈，涂片放置自然晾干。

4、细胞固定和破膜：用组化笔在玻片上涂抹了细胞的位置画圈（防止抗体流走），加 50-100μL 多聚甲醛固定液。室温固定 20min,后，洗掉固定液，加破膜工作液，室温孵育 20min，PBS 洗 3 次，每次 5 min。

5、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）。

6、加一抗：轻轻甩掉封闭液，滴加 PBS 按一定比例配好的一抗，玻片平放于湿盒内 4℃孵育过夜。

7、加二抗：玻片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覆盖细胞，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。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。

9、复染细胞核：苏木素复染 3min 左右，自来水洗，苏木素分化液分化数秒，自来水冲洗，苏木素返蓝液返蓝，流水冲洗。

10、脱水封片：将切片依次放入 75%酒精 5min--85%酒精 5min --无水乙醇Ⅰ5min --无水乙醇Ⅱ5min --二甲苯Ⅰ5min 中脱水透明，将切片从二甲苯拿出来稍晾干，中性树胶封片。

11、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 显出来的阳性信号为棕黄色。

Immunohistochemical Experiment Report of Cell Drop Tablet

I Experimental equipment and reagents

1.Experimental equipment

Name	Manufacturer	Model
Slide	Wanwu	Slide
Cover glass	Jiangsu Shitai Experimental Equipment Co., LTD.	10212432C
Vortex mixer	Wanwu	MX-F
Palm centrifuge	Wanwu	D1008E
Decolorization shaker	Wanwu	TSY-B
Pipette gun	Dragon	KE0003087/KA0056573
Tissue pencil	Wanwu	WG1066-1
Microscope	Nikon	E100
Imaging system	Japan Nikon	Nikon DS-U3

2.Main experimental reagents

Reagent	Manufacturer	Model	Dilution Ratio
Anhydrous ethanol	China National Pharmaceutical Group Chemical Reagent Co., LTD	100092683	
Xylene	Shanghai Lingfeng Chemical Reagent Co., LTD	1330-20-7	
Phosphate buffer saline	Wanwu	G0002	
Bovine serum albumin	Wanwu	G5001	
Rupture fluid	Wanwu	G1204	
4% Paraformaldehyde	Wanwu	G1101	
Neutral gum	China National Pharmaceutical Group Chemical Reagent Co., LTD	10004160	
Primary antibody:			
Secondary antibody:			

II Experimental procedure of immunocytochemistry

1. The cell suspension is centrifuged at 2800rpm at 4℃ for 5 minutes, the supernatant is discarded, and 2mL 4% Paraformaldehyde is added for fixation according to the amount of cells deposited at the bottom. If the cell pellet is not visible to the naked eye, it is centrifuged at 4℃ for 10 minutes at 3000rpm.
2. Take the cell suspension resuspended in 4% Paraformaldehyde,centrifuge at 2800rpm at 25℃ for 5 minutes, abandon the supernatant, according to the bottom of the precipitation to add PBS(PH7.4): ①If the cell pellet is not visible to the naked eye, it is centrifuged at 25℃ for 10 minutes at 3000rpm., when there is still no precipitation, leave about 0.2mL of liquid at the bottom, add 0.5mL of PBS and mix well, then use a pipette gun to suck up 200μL, and drop it into a small circle (3cm×2cm ellipse) drawn in advance with a tissue pencil; ②if the amount of cell precipitation is very small and the similar in size to mung beans, discard the supernatant, keep the bottom precipitation, add 0.5mL PBS, blow it with a pipette gun and mix it well, then absorb 200μL, and spread it in the small circle (3cm×2cm ellipse) drawn in advance with the composition; ③if the amount of cell precipitation is large and the size of soybean is similar or larger, the supernatant should be discarded and the bottom precipitation should be retained, add 2mL PBS, mix with a pipette gun, draw 150μL, and apply it in advance with a group pen in a large circle(maximum about 5cm long side, short edge of about 2cm of the elliptic), how much the amount of cells under a microscope, if it is still too thick, dilute the cell suspension double-fold with PBS until the amount of cells is evenly spread under the microscope
3. The cell suspension with pipette gun covered the whole circle, and place the section to dry naturally.
4. Cell fixation and membrane rupture: draw a circle on the position where the cells are smeared on the slide with a chemical pen (to prevent the antibody from flowing away), and add 50-100μL of 4% Paraformaldehyde. After fixation at room temperature for 20 minutes, wash away the fixative, add Rupture working fluid , incubate at room temperature for 20 minutes, and wash 3 times with PBS for 5 minutes each time.
5. 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).
6. 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℃.

7. 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.
8. 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.
9. Nucleus counterstaining: the sections are counterstained with hematoxylin stain solution for about 3 minutes; washed with tap water; differentiated with hematoxylin differentiation solution for several seconds; washed with tap water; treated with hematoxylin returning blue solution; washed with running water.
10. 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--xylene I for 5 minutes, dehydrated and transparent, remove the sections from xylene and let them dry slightly, then mount the sections with neutral gum.
11. 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.