石蜡切片免疫组化实验报告（Brdu）

一、实验器材及试剂

1、 实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
包埋机	武汉俊杰电子有限公司	JB-P5
病理切片机	上海徕卡仪器有限公司	RM2016
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	上海慧泰仪器制造有限公司	DHG-9140A
载玻片	Wanwu	
盖玻片	江苏世泰实验器材有限公司	10212432C
微波炉	格兰仕微波炉电器有限公司	P70D20TL-P4
脱色摇床	Wanwu	TSY-B
涡旋混合器	Wanwu	MX-F
掌上离心机	Wanwu	D1008E
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	
正丁醇	国药集团化学试剂有限公司	100052190	
盐酸	国药集团化学试剂有限公司	10011028	
柠檬酸（PH6.0)抗原修复液	Wanwu	G1202	
PBS 缓冲液	Wanwu	G0002	

4%多聚甲醛	Wanwu	G1101
3%双氧水	国药集团化学试剂有限公司	10011208
BSA	Wanwu	G5001
正常兔血清	Wanwu	G1209
苏木素染液	Wanwu	G1004
苏木素分化液	Wanwu	G1309
苏木素返蓝液	Wanwu	G1340
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、石蜡切片免疫组化实验步骤

1、石蜡切片脱蜡至水：依次将切片放入二甲苯I 15min--二甲苯II 15min--二甲苯 III 15min--无水乙醇I 5min--无水乙醇II 5min--85%酒精 5min--75%酒精 5min--蒸馏水洗。

2、抗原修复：组织切片置于盛满柠檬酸抗原修复缓冲液（PH6.0）的修复盒中于微波炉内进行抗原修复，中火 8min 至沸，停火 8min 保温再转中低火 7min，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。

3、DNA 变性：切片稍甩干后用组化笔在组织周围画圈（防止试剂流走），在组化圈内滴加提前配置好的的盐酸（盐酸：纯水=1:4），37℃温箱处理 30min，充分洗涤。

4、.阻断内源性过氧化物酶：切片放入 3%过氧化氢溶液（双氧水：纯水=1:9），室温避光孵育 25 min，将玻片置于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。

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，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I 5min --无水乙醇II 5min--正丁醇 5min --二甲苯I 5min 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、石蜡切片免疫组化结果判读

苏木素染细胞核为蓝色，DAB 显出的阳性表达为棕黄色。

Immunohistochemical Experiment Report of Paraffin Section (Brdu)

I Experimental equipments and reagents

1.Experimental equipments

Name	Manufacturer	Model
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan Junjie Electronics Co., LTD	JB-P5
Pathological slicer	Shanghai Leica Instrument Co., LTD	RM2016
Refrigerating table	Wuhan Junjie Electronics Co., LTD	JB-L5
Organizing spreader	Zhejiang Jinhua Kedi Instrument Equipment Co., LTD	KD-P
Oven	Shanghai Huitai Instrument Manufacturing Co., LTD	DHG-9140A
Slide	Wanwu	
Cover glass	Jiangsu Shitai Experimental Equipment Co., LTD	10212432C
Microwave oven	Galanz Microwave Oven Electrical Appliances co., LTD	P70D20TL-P4
Decolorization shaker	Wanwu	TSY-B
Vortex mixer	Wanwu	MX-F
Palm centrifuge	Wanwu	D1008E
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
N-butanol	China National Pharmaceutical Group Chemical Reagent Co., LTD	100052190
Hydrochloric acid	China National Pharmaceutical Group Chemical Reagent Co., LTD	10011028
Citric acid(PH6.0) antigen retrieval buffer	Wanwu	G1202
Phosphate buffer saline	Wanwu	G0002
4% Paraformaldehyde	Wanwu	G1101
3% Hydrogen peroxide	China National Pharmaceutical Group Chemical Reagent Co., LTD	10011208
Bovine serum albumin	Wanwu	G5001
Normal rabbit serum	Wanwu	G1209
Hematoxylin stain solution	Wanwu	G1004
Hematoxylin differentiation solution	Wanwu	G1309
Hematoxylin returning blue solution	Wanwu	G1340
Neutral gum	China National Pharmaceutical Group Chemical Reagent Co., LTD	10004160
Primary antibody: Secondary antibody: Immunohistochemical kit DAB chromogenic agent	Wanwu	G1211

II Immunohistochemical experimental procedure of paraffin section

1. Deparaffinzing and rehydrating the paraffin section: put the sections into xylene I for 15 minutes--xylene II for 15 minutes--xylene III for 15 minutes--absolute ethanol I for 5 minutes--absolute ethanol II for 5 minutes--85% alcohol for 5 minutes--75% alcohol for 5 minutes--rinse in distilled water.
2. Antigen retrieval: The tissue sections are placed in a repair box filled with citric acid(PH6.0)

antigen retrieval buffer for antigen retrieval in a microwave oven, heated on medium power for 8 minutes until boiling, then turned off the microwave oven, kept warm for 8 minutes and then transferred to medium-low power for heating 7minutes. During this process, excessive evaporation of buffer should be prevented and the sections should not be allowed to dry.To cool to room temperature before proceeding, the sections are placed in PBS(PH7.4) and shaken on the decolorization shaker 3 times for 5 minutes each.

3. DNA denaturation: after the sections are slightly dried, a tissue pencil is used to draw a circle around the tissue (to prevent the reagent from flowing away),and add pre-prepared hydrochloric acid (hydrochloric acid: pure water = 1:4) in the tissue circle. Treat in a 37℃ incubator for 30 minutes and wash thoroughly.
4. Blocking endogenous peroxidase activity: the sections are placed in 3% hydrogen peroxide (hydrogen peroxide: pure water=1:9) and incubated at room temperature in darkness for 25 minutes. The sections are placed in PBS(PH7.4) and shaken on a decolorizing shaper 3 times for 5 minutes each.
5. Serum sealing: 3%BSA is added to cover the tissues evenly within the circle, 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℃. (Add a small amount of water in the wet box to prevent evaporation of antibodies).
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 (HRP labeled) 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 5 minutes--anhydrous ethanol II 5 minutes--n-butanol 5 minutes--xylene I 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 immunohistochemical results of paraffin section

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