



冰冻切片 DAB 显色原位杂交 (CISH) 实验报告

1. 实验器材及试剂

1.1. 实验器材

名称	厂家	型号
冰冻切片机	Thermo	Cryotome E
载玻片	Servicebio	
盖玻片	江苏世泰实验器材有限公司	10212432C
无酶离心管	Servicebio	EP-150-M
摇床 (钟摆式)	Servicebio	TSY-B
涡旋混匀器	Servicebio	MV-100
移液枪	Dragon	KE0003087/KA0056573
免疫组化笔	Servicebio	G6100
冰箱	青岛海尔股份有限公司	BCD-192TGN
显微镜	CIC	XSP-C204
正置光学显微镜	日本尼康	NIKON ECLIPSE CI
成像系统	日本尼康	NIKON DS-U3
恒温箱	LABOTERY	GSP-70
高压灭菌锅	松下健康医疗	MLS-3751L-PC

1.2. 主要实验试剂

试剂	厂家	货号	稀释比
DEPC	Amresco	E174	
4%多聚甲醛 (DEPC 水)	Servicebio	G1113	
OCT 包埋剂	Servicebio	G6059-110ML	
无水乙醇	国药集团化学试剂有限公司	100092683	
环保型脱蜡透明液	Servicebio	G1128	
甲醇	国药集团化学试剂有限公司	40030561	
双氧水	国药集团化学试剂有限公司	10011208	
蔗糖	Servicebio	G5031	
PBS	Servicebio	G0002	
20×SSC 洗脱液	Servicebio	G3016-4	
BSA	Servicebio	G5001	

地址: 湖北省武汉市高新区高新二路 388 号生物医药加速器 C22 栋 5 楼 网址: <http://www.servicebio.cn>
电话: 4006-027-178

Add: 5th Floor, C22 Building, Biopark, No. 388 Gaoxin 2nd Road, East Lake High-tech Developing Zone,
Wuhan, Hubei, China, 430079 Website: <http://www.servicebio.com> Tel: 027-5111-3188



蛋白酶 K	Servicebio	G1234
苏木素染液	Servicebio	G1004
超净快干封片胶	Servicebio	G1404
杂交缓冲液	Servicebio	G3016-3
anti-DIG-HRP	jackson	200-032-156
DAB 显色剂	Servicebio	G1211

2. 冰冻切片 DAB 显色原位杂交实验步骤

- 2.1. **组织固定**: 组织取出洗净后立即放入 4%多聚甲醛 (DEPC 水配制) 固定 12h 以上。
- 2.2. **脱水**: 固定后组织放入 15%蔗糖溶液中 8h, 后换 30%蔗糖溶液中过夜。
- 2.3. **冰冻切片固定**: 冰冻切片室温晾干, 置于 4%多聚甲醛 (DEPC) 固定 10min, 于 PBS (PH7.4) 中在脱色摇床上晃动洗涤 3 次, 每次 5min。
- 2.4. **消化**: 基因笔画圈, 根据不同组织不同指标特性, 滴加蛋白酶 K (20ug/ml) 37° 消化____。纯水冲洗后 PBS 洗 3 次×5min。
- 2.5. **阻断内源性过氧化物酶**: 滴加 3%甲醇-H₂O₂, 室温避光孵育 15min, 将玻片置于 PBS (PH7.4) 中在脱色摇床上晃动洗涤 3 次, 每次 5min。
- 2.6. **预杂交**: 滴加预杂交液, 37° C 孵育 1h。
- 2.7. **杂交**: 倾去预杂交液, 滴加含探针____杂交液, 浓度____。恒温箱____度杂交过夜。
- 2.8. **杂交后洗涤**: 洗去杂交液, 2×SSC, 37° C 洗 10min, 1×SSC, 37° C 洗 2×5min, 0.5×SSC 室温洗 10min。若非特异杂交体较多, 可以增加甲酰胺洗涤。
- 2.9. **滴加封闭液**: 滴加封闭血清____正常兔血清____。室温 30min。
- 2.10. **滴加鼠抗地高辛标记过氧化物酶 (anti-DIG-HRP)**: 倾去封闭液, 滴加 anti-DIG-HRP。4 度 孵育过夜, 后 PBS 洗 4 次×5min。
- 2.11. **DAB 显色**: 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, 纯水冲洗切片终止显色。
- 2.12. **复染细胞核**: 苏木素复染 3min 左右, 自来水洗, 1%盐酸酒精分化数秒, 自来水洗, 氨水返蓝, 流水冲洗。
- 2.13. **脱水封片**: 将切片依次放入 75%酒精 6min—85%酒精 6min—100%酒精 I 6min—100%酒精 II 6min—正丁醇 6min—二甲苯透明, 将切片从二甲苯中拿出稍晾干后, 封片剂封片。
- 2.14. 显微镜检, 图像采集分析。

3. 冰冻切片 DAB 显色原位杂交实验结果判读

苏木素染细胞核为蓝色, DAB 显出的阳性表达为棕黄色。mRNA 原位杂交显示结果理论为胞浆阳性, 少数核阳性属正常。micRNA 与 lncRNA 不同指标表达定位不同。根据表达量显色有深浅。

注: 上述涉及到的所有试剂, 仪器等在 RNA 原位杂交实验时都需使用 DEPC 处理后的 Rnase



武汉赛维尔生物科技有限公司
Wuhan Servicebio Technology Co., Ltd.

free 环境。

附表 1 探针信息



Frozen Tissue-DIG-DAB-ISH Protocol

1. Apparatus and Reagents

1.1 Apparatus

Name	Producer	Model
Frozen slicer	Thermo	Cryotome E
Glass slide	Servicebio	
Coverslip	CITOTEST	10212432C
Enzyme-free centrifuge tubes	Servicebio	EP-150-M
Shaker	Servicebio	TSY-B
Vortex	Servicebio	MV-100
Pipettor	Dragon	KE0003087/KA0056573
Liquid Blocker PAP Pen	Servicebio	G6100
Refrigerator	HAIER	BCD-192TGN
Microscopy	Nikon	NIKON ECLIPSE CI
Imaging system	Nikon	NIKON DS-U3
Incubator	LABOTERY	GSP-70
autoclave	PANASONIC	MLS-3751L-PC

1.2 Major reagents

Reagent	Manufacturer	Article Number
DEPC	Amresco	E174
4% of paraformaldehyde (DEPC water)	Servicebio	G1113
OCT embedding agent	Servicebio	G6059-110ML
Ethanol	SCRC	100092683
BioDewax and Clear solution	Servicebio	G1128
Methanol	SCRC	40030561
Hydrogen peroxide	SCRC	10011208
Sucrose	Servicebio	G5031
PBS	Servicebio	G0002
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001



Proteinase K	Servicebio	G1234
Hematoxylin dye	Servicebio	G1004
SweSuper Clean BioMount Medium	Servicebio	G1404
Hybridization buffer	Servicebio	G3016-3
anti-DIG-HRP	Jackson	G3016-3
DAB reagent	Servicebio	G1211

2. The Steps of the Experiment

2.1. **Tissue fixation:** take out the organization, wash clean, then Immediately put in the fixed fluid (DEPC) above 12h.

2.2. **Tissue dehydration:** Transfer tissue to 15% sucrose solution for 8 h and were subsequently to a 30% sucrose solution overnight.

2.3. **Frozen section fixation:** The frozen sections were dried at room temperature, fixed in 4% paraformaldehyde (DEPC) for 10 min, and shaken on the shaker in PBS (pH 7.4) for three times, 5 min each.

2.4. **Digestion:** Mark the objective tissue with liquid blocker pen, according to the characteristics of different tissues and different indicators, add proteinase K (20 ug/ml) to cover tissues and incubate at 37°C for _____minutes. Washed with sterilized water and then washed three times in PBS for 5 min each time.

2.5. **Block endogenous peroxidase:** add 3% methanol-H₂O₂, incubate in dark at room temperature for 15min. Wash slides in PBS(PH7.4) three times for 5 min each with gentle agitation.

2.6. **Prehybridization:** add hybridization buffer onto specimen and incubate at 37°C for 1h.

2.7. **Hybridization:** remove the pre-hybridization solution, add the _____ probe hybridization solution with concentration of _____, and incubate the section in a humidity chamber and hybridize overnight at _____°C.

2.8. **Washing:** Remove the hybridization solution. Wash sections in 2×SSC for 10 min at 37°C , 1×SSC two times for 5 min each at 37°C, and 0.5×SSC for 10 min at room temperature. Formamide washing can be added if there are more non-specific hybrids.

2.9. **Blocking:** add blocking solution (Rabbit serum) to the section and incubate at room temperature for 30 min.

2.10. Add the mouse anti-digoxigenin-labeled peroxidase (anti-DIG-HRP): Remove the blocking solution and add anti-DIG-HRP. Incubate at 37 °C for 50 min, then Wash sections in PBS three times for 5 min each.

2.11. **DAB developing:** dry sections slightly, add fresh prepared DAB chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until positive expression appears



brown-yellow. Then stop developing reaction by wash in running tap water.

2.12. **Stain cell nuclei (counter stain):** Counterstain in nucleus with hematoxylin staining solution for 3 min and wash in tap water. Treat with the differentiate solution for a few seconds, wash in running tap water. Back to blue by bluing solution, wash in running tap water.

2.13. **Mounting:** Dehydrate successively in gradient ethanol of 75%, 85%, and 2 changes of pure ethanol, respectively, 6 min each. Clear in BioDewax and Clear solution for 6 min and mount with SweSuper Clean BioMount Medium.

2.14. Image the results using a brightfield fluorescence microscope. Store slides at RT.

3. Interpretation of the results

The nucleus stained with hematoxylin are blue, and the positive expression of DAB was brownish yellow. The results of in situ hybridization of mRNA were cytoplasmic positive and a few nuclear positive were normal. MicRNA and lncRNA were expressed differently. According to the expression of color depth.

Note: All reagents, instrument need RNase free.

Attached table 1 probe information.