



冰冻切片地高辛荧光原位杂交（TSA）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
正置荧光显微镜	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	G6059-110M	
		L	
无水乙醇	国药集团化学试剂有限公司	100092683	
二甲苯	国药集团化学试剂有限公司	10023418	
蔗糖	Servicebio	G5031	
PBS	Servicebio	G0002	
20×SSC 洗脱液	Servicebio	G3016-4	
BSA	Servicebio	G5001	
蛋白酶 K	Servicebio	G1234	
DAPI	Servicebio	G1012	
抗荧光淬灭封片剂	Servicebio	G1401	



anti-DIG-HRP	Jackson	200-032-156
FITC-Tyramide	Servicebio	G1222

2.冰冻切片地高辛荧光原位杂交 (TSA) 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。37°C 孵育 50min, 后 PBS 洗 3 次×5min。
- 2.11.滴加 FITC-Tyramide:** 滴加 FITC-Tyramide 试剂, 避光室温反应 5min。后 PBS 洗 3 次×5min。
- 2.12.DAPI 复染核:** 切片滴加 DAPI 染液, 避光孵育 8min, 冲洗后滴加抗荧光淬灭封片剂封片。
- 2.13.镜检拍照:** 切片于尼康正置荧光显微镜下观察并采集图像。(紫外激发波长 330-380nm, 发射波长 420nm, 发蓝光 FAM(488)绿光激发波长 465-495nm, 发射波长 515-555 nm, 发绿光; CY3 红光激发波长 510-560, 发射波长 590nm, 发红光。)

3.冰冻切片地高辛荧光原位杂交(TSA)实验结果判读

DAPI 染出来的细胞核在紫外的激发下为蓝色, 阳性表达为相应荧光素标记的荧光。FAM(488)为绿光, cy3 为红光。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。根据表达量不同荧光亮度有强弱。

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 free 环境。

附表 1 探针信息



Frozen Tissue-DIG-TSA-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
Xylene	SCRC	10023418
Sucrose	Servicebio	G5031
PBS	Servicebio	G0002
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001
Proteinase K	Servicebio	G1234
DAPI	Servicebio	G1012

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



Anti-fluorescence quenching sealing tablets	Servicebio	G1401
anti-DIG-HRP	Jackson	200-032-156
FITC-Tyramide	Servicebio	G1222

2. The steps of the experiment

2.1. **Tissue fixation:** tissue was washed and fixed with 4% paraformaldehyde for 12 h or more.

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) three times for 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 with 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. Tyramide **developing:** dry sections slightly, add fresh prepared Tyramide chromogenic reagent to marked tissue. Reaction in dark for 5 min at room temperature. Then wash sections in PBS three times for 5 min each.

2.12. **Stain cell nuclei (counter stain):** Incubate with DAPI for 8min in the dark, and then mounting with anti-fluorescence quenching sealing tablets.



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Wuhan Servicebio Technology Co., Ltd.

2.13. Microscopic examination and photography : To take photos with positive fluorescence microscope. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; FAM glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm; CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nm.

3. Interpretation of the results

The nuclear stained by DAPI were blue under ultraviolet excitation, and the positive expression was a kind of fluorescence labeled by corresponding luciferin. FAM (488) appears green, cy3 appears red. The results of mRNA in situ hybridization were cytoplasmic positive and a few nuclear positive were normal. MicRNA and lncRNA were expressed differently. According to the expression, Different fluorescence brightness is strong or weak.

Note: All reagents, instrument need RNase free.

Attached table 1 probe information.