

石蜡切片 SABER-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
脱水机	DIAPATH	Donatello
载玻片	wanwu	
盖玻片	江苏世泰实验器材有限公司	10212432C
病理切片机	上海莱卡仪器有限公司	RM2016
无酶离心管	wanwu	EP-150-M
摇床(钟摆式)	wanwu	TSY-B
涡旋混匀	wanwu	MX-F
移液枪	Dragon	KE0003087/KA0056573
Gene tech pen	Gene tech	GT1001
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
包埋机	武汉俊杰电子有限公司	JB-P5
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

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)	wanwu	G1113	
石蜡	Sakura		
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	
PBS 缓冲液 (DEPC)	wanwu	G0002	
20×SSC 洗脱液	wanwu	G3016-4	
BSA	wanwu	G5001	
蛋白酶 K	wanwu	G1205	
DAPI	wanwu	G1012	

抗荧光淬灭封片剂	wanwu	G1401
杂交缓冲液	wanwu	G3016-3
anti-DIG-HRP	Jackson	
FITC-TSA	wanwu	200-032-156

2.石蜡切片地高辛荧光原位杂交实验步骤

- 2.1.组织固定:** 组织取出洗净后立即放入固定液 (DEPC 水配制) 中固定 12h 以上。
- 2.2.脱水:** 组织固定完成后经梯度酒精脱水后浸蜡, 包埋。
- 2.3.切片:** 石蜡经切片机制片, 摊片机捞片, 62°烤箱烤片 2h。
- 2.4.石蜡切片脱蜡至水:** 依次将切片放入二甲苯I15min-二甲苯II15min-无水乙醇I5min-无水乙醇II5min, 风干, DEPC 水浸泡。。
- 2.5.消化:** 根据组织固定时间长短, 切片于修复液中煮沸 10 分钟, 自然冷却。后基因笔画圈, 根据不同组织不同指标特性, 滴加蛋白酶 K(20ug/ml) 37°消化 20 min。纯水冲洗后 PBS 洗 3 次×5min。
- 2.6.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。
- 2.7.预杂交:** 滴加预杂交液, 37°C 孵育 1h。
- 2.8.杂交:** 倾去预杂交液, 滴加含探针 hsa_circ_0061140 杂交液, 浓度 500nM, 恒温箱 42 度杂交过夜。
- 2.9.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。
- 2.10.二标孵育:** 滴加含二标探针杂交液, 稀释比 1: 400.42° 孵育 3h。后 2×SSC, 37°C 洗 10min, 1×SSC, 37°C 洗 2×5min, 0.5×SSC 37°洗 10min。
- 2.11.滴加封闭液:** 滴加封闭血清 正常兔血清。室温 30min。
- 2.12.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。
- 2.13.滴加 FITC-TSA:** 滴加 FITC-TSA 试剂, 避光室温反应 5min。后 TBST 洗 3 次×10min.PBS 洗 1 次×5min。
- 2.14.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。
- 2.15.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.石蜡切片地高辛荧光原位杂交实验结果判读

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 (488) 标记的绿光。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

hsa_circ_0061140 probe:

5 '-AACTTCACTCCACACTTCTGAGAAAAATGTGA

(ttt CATCATCAT ACATCATCAT) ₃₀ -3'

二标探针:

5' -DIG-tt ATGATGATGT ATGATGATGT -3'

Paraffin –DIG -TSA -ISH protocol**1. Apparatus and reagents****1.1 Apparatus**

Name	Producer	Model
Dehydrator	DIAPATH	Donatello
Glass slide	wanwu	
Coverslip	Citotest	10212432C
Pathologic microtome	Leica	RM2016
Enzyme-free centrifuge tubes	wanwu	EP-150-M
Shaker	wanwu	TSY-B
Vortex	wanwu	MX-F
Pipettor	Dragon	KE0003087/KA0056573
Gene tech pen	Gene tech	GT101
Microscopy	Nikon	NIKON ECLIPSE CI
Imaging system	Nikon	NIKON DS-U3
Incubator	LABOTERY	GSP-70
autoclave	PANASONIC	MLS-3751L-PC
Paraffin embedding machine	WHJJ	JB-P5
Frozen flat	WHJJ	JB-L5
Tissue spreader	Kedee	KD-P

1.2 Major reagents

reagent	manufacturer	article number
DEPC	Amresco	E174
4% of paraformaldehyde (DEPC water)	wanwu	G1113
Paraffin wax	Sakura	G1113
Ethanol	SCRC	100092683
Xylene	SCRC	10023418
PBS solution (DEPC)	wanwu	G0020
20×SSC solution	wanwu	G3016-4
BSA	wanwu	G5001

Proteinase K	wanwu	G1205
DAPI	wanwu	G1012
Anti-fluorescence quenching sealing tablets	wanwu	G1401
hybridization buffer	wanwu	G3016-3
anti-DIG-HRP	Jackson	G3016-3
FITC-TSA	wanwu	200-032-156

2. The steps of the experiment

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

2.2 **Dehydration:** The tissue is dehydrated by gradient alcohol, paraffin, embedding.

2.3 **Section:** the paraffin is sliced through the slicer, the piece of the slice machine and the 62 - degree oven roast for 2 hours.

2.4 **Dewaxing and dehydration:** soak sections in 2 changes of xylene, 15 minutes each. Dehydrate in 2 changes of pure ethanol for 5 minutes each. Then, followed respectively by dehydrating in gradient ethanol of 85%and 75% ethanol 5 minutes for each. Wash in DEPC dilution.

2.5 **Digestion:** according to the tissue fixation time, the slices are boiled in the retrieval solution for 10-15 minutes and naturally cooled. Mark the objective tissue with liquid blocker pen, according to the characteristics of tissues, Add proteinase K(20 ug/ml) working solution to cover objectives and incubate at 37°C for ___ min. Washing with pure water, then wash three times with PBS (pH 7.4) in a Rocker device, 5 min each.

2.6 **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.

2.7 **Pre-hybridization:** add Pre-hybridization solution to each section and incubate for 1 h at 37°C.

2.8 **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.

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

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

2.11 Add the mouse anti-digoxigenin-labeled peroxidase (anti-DIG-HRP): remove the blocking solution and add anti-DIG-HRP. Incubate at 37 °C for 40 min, then Wash sections in PBS four times for 5 min each.

2.12 **TSA developing:** dry sections slightly, add fresh prepared TSA chromogenic reagent to marked tissue. Reaction in dark for 5 min at room temperature. Then wash sections in PBS three times for 10 min each.

2.13 **Stain cell nuclei (counter stain):** incubate with DAPI for 8min in the dark, and then mounting.

2.14 **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.