

细胞爬片荧光探针原位杂交双标 (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
盖玻片	江苏世泰实验器材有限公司谷歌生物	10212432C
无酶离心管	Wanwu	EP-150-M
摇床(钟摆式)	Wanwu	TSY-B
涡旋混匀器	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
倒置荧光显微镜	日本尼康	NIKON ECLIPSE TI-SR
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)	Wanwu	G1113	
无水乙醇	国药集团化学试剂有限公司	100092683	
PBS 缓冲液(DEPC)	Wanwu	G0020	
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	Jackson	G1401	
anti-DIG-HRP	Wanwu	200-032-156	
TSA-FITC			

2. 细胞爬片荧光探针原位杂交实验步骤

2.1. 细胞爬片固定: 细胞爬片置于 4%多聚甲醛 (DEPC) 固定 20min, 于 PBS (PH7.4) 中在脱色摇床上晃动洗涤 3 次, 每次 5min。

2.2. 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) 消化 8min。

纯水冲洗后 PBS 洗 3 次×5min。 .

2.3.预杂交: 滴加预杂交液 37°恒温箱 1h。

2.4.杂交: 倾去预杂交液, 滴加杂交液 (含探针____浓度 ____), ____度杂交过夜。

2.5.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 37°洗 10min。若非特异杂交体较多, 可以增加甲酰胺洗涤

2.6.滴加封闭液: 滴加封闭血清__正常兔血清__。室温 30min。

2.7.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。

2.8.滴加 FITC-TSA: 滴加 FITC-TSA 试剂, 避光室温反应 5min。后 TBST 洗 3 次×5min.PBS 洗 1 次×5min。

2.9.杂交: 倾去预杂交液, 滴加杂交液 (含探____浓度 ____), ____度杂交过夜。

2.10.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 37°洗 10min。若非特异杂交体较多, 可以增加甲酰胺洗涤

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 CY3-TSA: 滴加 CY3-TSA 试剂, 避光室温反应 5min。后 TBST 洗 3 次×5min.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.细胞爬片荧光探针原位杂交双标实验结果判读

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

Cell climbing –DIG -TSA double fluorescent -ISH protocol**1. Apparatus and reagents****1.1 Apparatus**

Name	Producer	Model
Coverslip	Citotest	10212432C
Enzyme-free centrifuge tube	Wanwu	EP-150-M
Shaker	Wanwu	TSY-B
Vortex	Wanwu	MX-F
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker pen	Gene tech	GT1001
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)	Wanwu	G1113
Ethanol	SCRC	100092683
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. cell climbing fixation: cell climbing was fixed in 4% paraformaldehyde (DEPC) for 20 min, washed 3 times with PBS (pH 7.4) on a decolorizing shaker for 5 min each time.

2.2. 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, then washed three times with PBS, 5 min each time

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

2.4. **Hybridization:** remove the pre-hybridization solution, add the _____ probe hybridization solution with concentration of____, and incubate the cell climbing in a humidity chamber and hybridize overnight at ____°C.

2.5. **Washing:** Remove the hybridization solution. Wash sections with 2×SSC for 5 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.

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

2.7. 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.

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

2.9 **Hybridization:** remove the pre-hybridization solution, add the _____ probe hybridization solution with concentration of____, and incubate the cell climbing in a humidity chamber and hybridize overnight at ____°C.

2.10 **Washing:** Remove the hybridization solution. Wash sections with 2×SSC for 5 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.

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

2.12 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.

2.13 **CY3-TSA developing:** dry sections slightly, add fresh prepared CY3-TSA chromogenic reagent to marked tissue. Reaction in dark for 5 min at room temperature. Then wash sections in TBST three times for 10 min each and use PBS wash for 5 min.

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

2.15 **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.