



细胞爬片 SweAMI-荧光原位杂交 (FISH) +IF 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	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	
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	
杂交缓冲液	Servicebio	G3016-3	
一抗⑦			
二抗⑧			

2.细胞爬片 SweAMI 荧光原位杂交+IF 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。

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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②min。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 ③浓度 ④), ⑤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. 杂交 2: 轻轻甩干切片, 滴加已预热的探针混合物 2 杂交液 (60 μL), 水平置于湿盒内 40°C 杂交 45 min。此过程中在湿盒底部加入 50ml 2× SSC, 防止干片。

2.7. 杂交后洗涤: 倾去杂交液, 将切片依次用 40° C 预热的 2× SSC、1× SSC、0.5× SSC、0.1× SSC 于 40° C 漂洗 5 min。注: 若非特异性杂交体较多, 可以在此步增加洗涤时间、次数及调整杂交液中甲酰胺浓度。

2.8. 信号杂交: 轻轻甩干切片, 滴加已预热的信号探针杂交液 (60 μL), 水平置于湿盒内 40°C 杂交 45 min。此过程中在湿盒底部加入 50 ml 2× SSC, 防止干片。

2.9. 杂交后洗涤: 倾去杂交液, 将切片依次用 40° C 预热的 2× SSC、1× SSC、0.5× SSC、0.1× SSC 于 40°C 漂洗 5 min。注: 若非特异性杂交体较多, 可以在此步增加洗涤时间、次数及调整杂交液中甲酰胺浓度。

2.10. 滴加封闭液: 滴加封闭血清 ⑥。室温 30min。

2.11. 孵育一抗: 滴加一抗 ⑦, PBS 稀释比 ⑨。4°过夜。后 PBS 洗 3×5min。

2.12.孵育二抗: 滴加相应二抗 ⑧, 室温孵育 50min。后 PBS 洗 3×5min。

2.13.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。

2.14.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 SweAMI 荧光原位杂交+IF 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。Cy3 标记的红光。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 –SweAMI-FISH+IF Protocol

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Coverslip	Citotest	10212432C
Enzyme-free centrifuge tube	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
Ethanol	SCRC	100092683
PBS	Servicebio	G0002
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001
Proteinase K	Servicebio	G1234
DAPI	Servicebio	G1012
Anti-fluorescence quenching sealing tablets	Servicebio	G1401
hybridization buffer	Servicebio	G3016-3
anti-DIG-HRP	Jackson	G3016-3
FITC-Tyramide	Servicebio	G1222
First antibody		
Second antibody		



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 section 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. **Hybridization2:** Discard the solution, add the probe2 hybridization solution 60ul, and incubate the section in a humidity chamber and hybridize overnight at 40°C.

2.7. **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.

2.8. **Signal Hybridization:** Discard the solution, add the signal probe hybridization solution 60ul, and incubate the section in a humidity chamber and hybridize overnight at 40°C.

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

2.10. **Incubate first antibody:** PBS solution containing a _____ dilution of primary antibody were added and incubated at 4°C overnight. Samples were then washed with PBS three times for 5 min each at RT.

2.11. **Incubate second antibody:** after washing, the section was incubated for 50 min with second antibody at RT. Samples were then washed with PBS three times for 5 min each at RT.

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

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



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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.

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