# 细胞爬片荧光探针原位杂交（FISH）+免疫荧光（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 | 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 | Wanwu | G1401 | |
| 杂交缓冲液 | Wanwu | G3016-3 | |
| 一抗 | | | |
| 二抗 | | | |

## 2.细胞爬片荧光探针原位杂交+免疫荧光实验步骤

**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 1-5min。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：**待爬片完全冷却后，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_____。室温 30min。

**2.7.孵育一抗：**滴加一抗_____，PBS 稀释比_____。4°过夜。后 PBS 洗 3×5min。

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

**2.9.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。

**2.10.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+免疫荧光实验结果判读**

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。免疫荧光结果根据不同指标，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 –fluorescence probe-FISH and Immunofluorescence 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 |
| 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, wash 3 times with PBS (pH 7.4) in 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℃ for_____minutes. wash in sterilized water，then washed three times in PBS，5 min each.

2.3. **Prehybridization:** add hybridization buffer onto specimen and incubate at 37℃ 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 ___℃.

2.5. **Washing**: remove the hybridization solution. Wash sections in 2×SSC for 5 min at 37℃ ，wash sections in 1×SSC two times for 5 min each at 37℃， 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 serum to the section and incubate at room temperature for 30 min.

2.7. **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.

2.8. **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.

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

2.10 **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.**