# 细胞爬片 BCIP/NBT 显色原位杂交（CISH）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 |
| 显微镜 | CIC | XSP-C204 |
| 正置光学显微镜 | 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 | |
| 无水乙醇 | 国药集团化学试剂有限公司 | 100092683 | |
| PBS | Servicebio | G0002 | |
| 20×SSC 洗脱液 | Servicebio | G3016-4 | |
| BSA | Servicebio | G5001 | |
| 蛋白酶 K | Servicebio | G1234 | |
| 核固红染液 | Servicebio | G1035 | |
| 中性树胶 | 国药集团化学试剂有限公司 | 10004160 | |
| 杂交缓冲液 | Servicebio | G3016-3 | |
| Anti-DIG-AP | jackson | 200-052-156 | |
| NBT/BCIP 显色剂 | 博士德 | AR1023 | |

## 2.细胞爬片 BCIP/NBT 显色原位杂交实验步骤

**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 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：**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__BSA____。室温 30min。

**2.7.滴加鼠抗地高辛标记碱性磷酸酶（anti-DIG-AP）：**倾去封闭液，滴加 anti-DIG-AP。37°C 孵育 40min，后 TBS 洗 4 次×5min。

**2.8.BCIP/NBT 显色：**滴加 BCIP/NBT 显色液，显微镜观察阳性。后纯水冲洗。

**2.9.复染细胞核：**滴加核固红染液，染核至合适程度后纯水冲洗。

**2.10.封片：**自然风干后，中性树胶封片。

**2.11.**显微镜检，图像采集分析。

**3.细胞爬片 BCIP/NBT 显色原位杂交实验结果判读**

核固红染细胞核为红色，BCIP/NBT 显出的阳性表达为蓝紫色。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。根据表达量显色有深浅。

**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**

# DAB /BCIP DIG-ISH Protocol（Cell Climbing）

## 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 |
| 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 |
| Nuclear fast red dye | Servicebio | G1035 |
| Glycerin-gelatin | Servicebio | G1402 |
| Hybridization buffer | Servicebio | G3016-3 |
| anti-DIG-AP | Jackson | 200-052-156 |
| NBT/BCIP reagent | Boster | AR1023 |

**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℃ 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℃ 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___℃.

2.5. **Washing**: remove the hybridization solution. Wash sections with 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 solution (Rabbit serum) to the section and incubate at room temperature for 30 min.

2.7. **Add the Alkaline Phosphatase-conjugated IgG Fraction Monoclonal Mouse Anti-Digoxin Antibody:** (anti-DIG-AP): Remove the blocking solution and add anti-DIG-HRP. Incubate at 37 ℃ for 40 min, then Wash sections in TBS four times for 5 min each.

2.8 **NBT/BCIP developing**: dry sections slightly, add fresh prepared NBT/BCIP chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until positive expression appears shows blue-purple. Then stop developing reaction by wash in running tap water.

2.9. **Multicolor nucleus:** dripping with red dye, and then rinse with pure water.

2.10 **Mounting:** Natural air dry and mount with Glycerol Jelly mounting medium.

2.11 **Image the results using a brightfield fluorescence microscope**. Store slides at RT.

**3. Interpretation of the Results**

The nucleus stained with hematoxylin are blue, and the positive expression of DAB was brownish yellow. The results of in situ hybridization of mRNA were cytoplasmic positive and a few nuclear positive were normal. MicRNA and lncRNA were expressed differently. According to the expression of color depth.

Note: All reagents, instrument need RNase free.

**Attached table 1 probe information.**