



石蜡切片 SweAMI-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
脱水机	DIAPATH	Donatello
载玻片	Servicebio	
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	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
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)	Servicebio	G1113	
石蜡	Sakura		
无水乙醇	国药集团化学试剂有限公司	100092683	
环保型脱蜡透明液	Servicebio	G1128	
PBS	Servicebio	G0002	
20×SSC 洗脱液	Servicebio	G3016-4	
BSA	Servicebio	G5001	

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蛋白酶 K	Servicebio	G1234
核固红染液	Servicebio	G1035
超净快干封片胶	Servicebio	G1404
杂交缓冲液	Servicebio	G3016-3
anti-DIG-AP	jackson	200-052-156
BCIP/NBT 显色剂	博士德	AR1023

2. 石蜡切片 BCIP/NBT 显色原位杂交实验步骤

- 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：**石蜡经切片机切片，摊片机捞片，62°烤箱烤片 2h。
- 2.4.石蜡切片脱蜡至水：**依次将切片放入脱蜡透明液I15min-脱蜡透明液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___min。纯水冲洗后 PBS 洗 3 次×5min。
- 2.6.预杂交：**滴加预杂交液，37°C 孵育 1h。
- 2.7.杂交：**倾去预杂交液，滴加含探针____杂交液，浓度___500nM___，恒温箱___42___度杂交过夜。
- 2.8.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。
- 2.9. Imaging oligo (DIG)孵育：**滴加含 Imaging oligo (DIG)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。
- 2.10.滴加封闭液：**滴加封闭血清___正常兔血清___。室温 30min。
- 2.11.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 50min，后 TBS 洗 4 次×5min。
- 2.12.BCIP/NBT 显色：**滴加 BCIP/NBT 显色液，显微镜观察阳性。后纯水冲洗。
- 2.13.复染细胞核：**滴加核固红染液，染核至合适程度后纯水冲洗。
- 2.14.封片：**自然风干后，超净快干封片胶封片。
- 2.15.显微镜检，图像采集分析。**

3. 石蜡切片 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。



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附表 1 探针信息



NBT/BCIP-ISH Assay Protocol (Paraffin Slide)

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Dehydrator	WHJJ	JJ-12J
Paraffin embedding machine	WHJJ	JB-P5
Pathologic microtome	Leica	RM2016
Frozen flat	WHJJ	JB-L5
Water Bath-Slide	Kedee	KD-P
RNase-freeGlass microscope slides	Servicebio	
Micro-centrifuge	Servicebio	D1008E
Rocker	Servicebio	TSY-B
Vortex	Servicebio	MV-100
Pipettor	Dragon	KE0003087/KA0056573
Microscopy	CIC	XSP-C204
Liquid Blocker PAP Pen	Servicebio	G6100
Refrigerator	HAIER	BCD-192TGN
incubator	LABOTERY	GSP-70
autoclave	PANASONIC	MLS-3751L-PC

1.2 Major reagents

reagent	manufacturer	article number
Ethanol	SCRC	100092683
BioDewax and Clear solution	Servicebio	G1128
PBS	Servicebio	G0002
Proteinase K	Servicebio	G1234
4% of paraformaldehyde (DEPC water)	Servicebio	G1113
DEPC	Amresco	E174
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001
Nuclear fast red dye	Servicebio	G1035
Glycerin-gelatin	Servicebio	G1402

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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 Organization fixed: Take out the organization, wash clean, then Immediately put in the fixed fluid (DEPC) to fix 2-12h.

2.2 Dehydration: The tissue is dehydrated by gradient alcohol and then paraffin embedded.

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 Dewaxing Transparent Liquid, 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 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(20ug/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 Pre-hybridization: Add Pre-hybridization solution to each section and incubate for 1 h at 37°C.

2.7 Hybridization: Discard the pre-hybridization solution, add the probe hybridization solution ,concentration___, and incubate the section in a humidity chamber and hybridize overnight at___°C.

2.8 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.

2.9 Imaging oligo (DIG): Add the hybridization solution containing Imaging Oligo (DIG), Dilution ratio 1:400. 42 ° FOR 3 hours. 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.

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

2.11 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 °C for 40 min, then Wash sections in TBS four times for 5 min each.



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2.12 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.

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

2.14 Mounting: Natural air dry and mount with SweSuper Clean BioMount Medium.

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

3. Interpretation of the results

The nuclei were red, and the positive expression of BCIP/NBT was blue-purple. The results of mRNA in situ hybridization showed that cytoplasm was positive and a few nuclear positive were normal. The expression of micRNA and lncRNA was different. According to the expression, the color is very light.

Attached table 1 probe information.