



细胞爬片 DAB 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 储存液	Servicebio	G1234	
苏木素染液	Servicebio	G1004	
超净快干封片胶	Servicebio	G1404	
杂交缓冲液	Servicebio	G3016-3	
anti-DIG-HRP	jackson	200-032-156	
DAB 显色剂	Servicebio	G1211	

2. 细胞爬片 DAB 显色原位杂交实验步骤

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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过氧化物酶（anti-DIG-HRP）：倾去封闭液，滴加 anti-DIG-HRP。37°C 孵育 40min，后 PBS 洗 4 次×5min。

2.8.DAB 显色：切片稍甩干后，在圈内滴加新鲜配制的 DAB 显色液，显微镜下控制显色时间，阳性为棕黄色，纯水冲洗切片终止显色。

2.9.复染细胞核：苏木素复染 3min 左右，自来水洗，1%盐酸酒精分化数秒，自来水洗，氨水返蓝，流水冲洗。

2.10.脱水封片：将切片依次放入 75%酒精 6min-85%酒精 6min—100%酒精 I 6min—100%酒精 II 6min-正丁醇 6min，晾干后，超净快干封片胶封片。

2.11.显微镜检，图像采集分析。

3.细胞爬片 DAB 显色原位杂交实验结果判读

苏木素染细胞核为蓝色，DAB 显出的阳性表达为棕黄色。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



Paraffin-DIG-DAB-ISH Protocol

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
Xylene	SCRC	10023418
PBS	Servicebio	G0002
Proteinase K	Servicebio	G1234
4% of paraformaldehyde (DEPC water)	Servicebio	G1113
Methanol	SCRC	
Hydrogen peroxide	SCRC	
DEPC	Amresco	E174
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001
Hematoxylin dye	Servicebio	G1004

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SweSuper Clean BioMount Medium	Servicebio	G1404
hybridization buffer	Servicebio	G3016-3
anti-DIG-HRP	Jackson	
DAB reagent	Servicebio	G1211

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, paraffin, Embedding.

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 xylene, 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 Block endogenous peroxidase: Add 3% methanol-H₂O₂ , incubate in dark at room temperature for 15min. Wash slides in PBS (pH 7.4) three times for 3 min each, with gentle agitation and protected from light.

2.7 Pre-hybridization: Add Pre-hybridization solution to each section and incubate for 1 h at 37°C.

2.8 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.

2.9 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.

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

2.11 Add the mouse anti-digoxigenin-labeled peroxidase (anti-DIG-HRP): Remove the blocking solution and add anti-DIG-HRP. Incubate at 37 °C for 40 min, then wash sections in PBS four times for 5 min each.



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2.12 DAB developing: Dry sections slightly, add fresh prepared DAB chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until positive expression appears brown-yellow. Then stop developing reaction by wash in running tap water.

2.13 Counterstain in nucleus with Hematoxylin staining solution for 3 min and wash in tap water. Treat with the differentiate solution for a few seconds, wash in running tap water. Back to blue by bluing solution, wash in running tap water.

2.14 Dehydrate successively in gradient ethanol of 75%, 85%, and 2 changes of pure ethanol, respectively, 6 min each. 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 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.