PolyBranch HCR™ miRNA、circRNA FISH Kit(Plant)

Catalog Number:HKR13Z

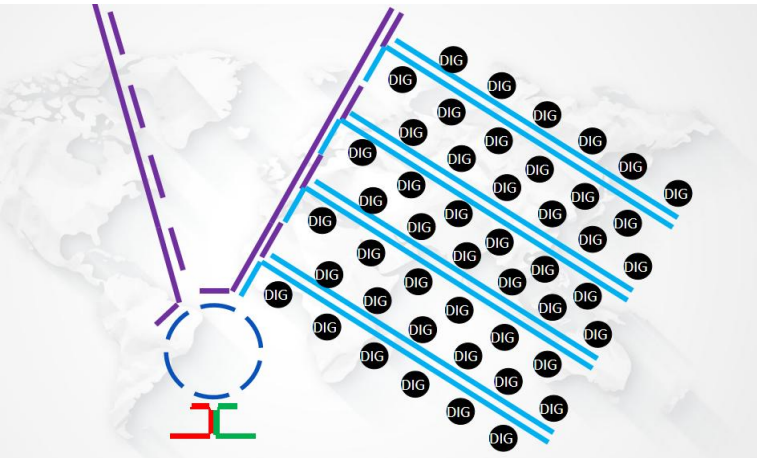
【Product Information】

Product Name	Catalog No	20T	Storage	Shelf Life
Wash Buffer (powder, dissolve in 10L ddH2O)	HKR13-1	10L	Short-term:4℃ Long-term:-20℃	12 months
Proteinase K（100x）	HKR13-2	20uL		
Pectinase/Cellulase Mixture	HKR13-3	2mL		
AP-Mouse Anti-Digoxin (100x)	HKR13-4	20uL		
NBT Chromogen Solution A (20x)	HKR13-5	100uL		
NBT Chromogen Solution B (20x)	HKR13-6	100uL		
Blocking Buffer	HKR13-7	2mL		
Probe:	HKR13-8	2mL		
Pre-Amplification Probe	HKR13-9	2mL		
Amplification Probe	HKR13-10	2mL		

【Product Description】

The miRNA hybridization kit includes a combination probe, a pre amplification structure, and an amplification probe. When two probes bind to adjacent positions of the target sequence, a small segment of the top sequence can bind to the pre amplification structure. The repeated

sequence on the pre amplification structure can trigger a branched HCR to form a large nucleic acid aggregate. Each nucleic acid strand is labeled with digoxin, and the antibody recognizes digoxin for color development.



【Probe Information】

【Tissue Fixation】

Tissue Type	Animal	Plant	Frozen Samples	Cell Climb Slides	Cells
Treatment	Fix at RT for 12h, paraffin embed	Vacuum fix for 1h, RT fix for 12h, paraffin embed.	Dehydrate in 15% sucrose at 4℃ for 8h,then in 30% sucrose at 4℃ for 8h, OCT embed.	Fix at 4℃ for 2h.	scrape off cells,fix in 4% PFA at 4℃ for 2h, wash with PBS, agarose embed.
Type	mRNA	lncRNA	circRNA	miRNA	rRNA
Treatment	Fix at RT for 12h	Fix at RT for 12h (<300bp: 24h)	Fix at RT for 12h	Fix at RT for 12h	Fix at RT for 12h (<300bp: 24h)

【Storage and Shipping】

Ship on wet ice; store at -20℃ for long-term or at 4℃ for short-term use. Shelf life: 6 months.

【Protocol】

1. Deparaffinization and Rehydration

Immerse slides sequentially in:Xylene I (15 min) → Xylene II (15 min) → Xylene III (15 min) → 100% ethanol (10 min) → 90% ethanol (10 min) → 80% ethanol (10 min) → 70% ethanol (10 min) → Rinse with distilled water.

2. Enzyme Repair

After slides are completely dry, draw a hydrophobic circle around the tissue using a histology pen (recommended: HKR14P In Situ Hybridization Pen). Place slides horizontally in a hybridization oven or humidified chamber. Add 100 μL of Pectinase/Cellulase Mixture onto the tissue and incubate at 37℃ for 30 min. Rinse with distilled water to stop the reaction. Remove excess liquid, then add 100 μL of Proteinase K repair solution (1X) and incubate at 37℃ for 30 min. Rinse with distilled water to stop the reaction.

3. Blocking

Remove excess liquid from slides. Add 100 μL of Blocking Buffer per slide and incubate at 37℃ for 30 min. Wash once for 5 min.**Wash steps: Place slide racks in a wash tank, add wash buffer (ensure samples are submerged), and shake at 60 rpm for 5 min.**

4. Probe Hybridization

Remove excess liquid from slides. Add 100 μL of probe per slide and incubate at 37℃ for 3 h or overnight (maintain humidity to prevent drying). Wash 5 times, 5 min each.**Wash steps: As described above.**

5. Pre-Amplification Hybridization

Remove excess liquid from slides. Add 100 μL of Pre-Amplification Probe to each slide

and incubate at 37 ℃ for 3 h or overnight (ensure humidity). Wash 5 times, 5 min each.**Washing steps: As described above.**

6. Probe Amplification

Remove excess liquid from slides. Add 100 µL of Amplification Probe per slide and incubate at 37 ℃ for 1.5 h (maintain humidity). Wash 5 times, 5 min each.**Wash steps: As described above.**

7. AP-Mouse Anti-Digoxin

Remove excess liquid from slides. Add 100 µL of AP-Mouse Anti-Digoxin (1X) per slide and incubate at 37 ℃ in a humidified chamber for 40 min. Wash 5 times, 5 min each.**Wash steps: As described above.**

8. Chromogenic Reaction

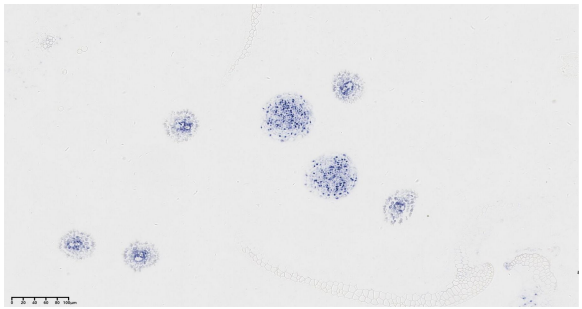
Prepare the chromogenic solution by adding 50 µL of NBT Chromogen Solution A and 50 µL of NBT Chromogen Solution B to 1 mL of deionized water. Add 100 µL of the mixture per slide and incubate at RT for 30 min. Rinse with distilled water to stop the reaction. Air-dry slides and mount with mounting medium.

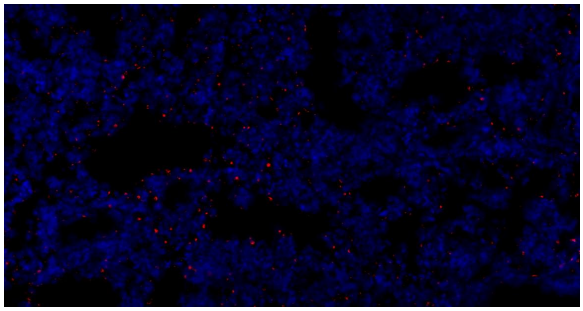
【Precautions】

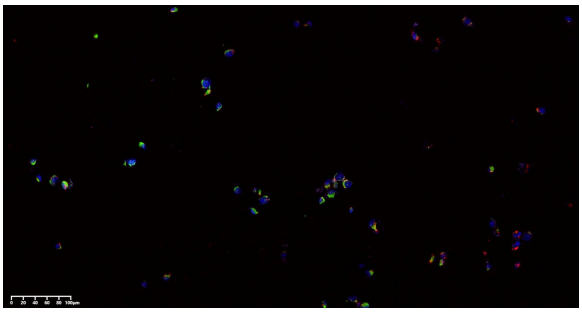
1. For research use only.

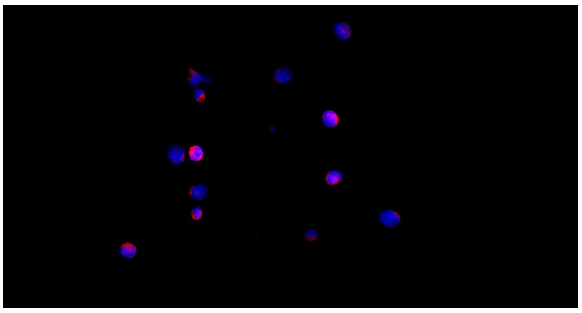
2. Wear lab coats and disposable gloves for safety.

【Example Images】









PolyBranch™ miRNA 、circRNA FISH Kit（植物）

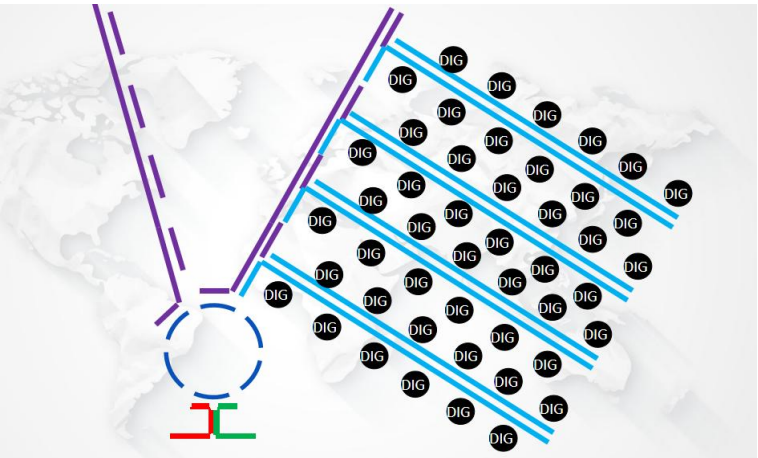
货号：HKR13Z

【产品信息】

产品名称	产品货号	20T	保存	有效期
洗液（粉末溶解于 10L ddH2O）	HKR13-1	10L	短期4℃ 长期 -20℃	12 个月
蛋白酶 K（100x）	HKR13-2	20uL		
果胶酶纤维素酶混合液	HKR13-3	2mL		
AP-鼠抗地高辛（100x）	HKR13-4	20uL		
NBT 显色液 A（20x）	HKR13-5	100uL		
NBT 显色液 B（20x）	HKR13-6	100uL		
封闭液	HKR13-7	2mL		
探针	HKR13-8	2mL		
预放大探针	HKR13-9	2mL		
放大探针	HKR13-10	2mL		

【产品简介】

miRNA 杂交试剂盒，包含组合式探针、预放大结构、放大探针，当两个探针结合在目标序列相邻的位置上时，顶部的一小段序列可以结合预放大结构，预放大结构上的重复序列可以触发树叉状 HCR 形成了一个大分子核酸聚集体，每条核酸链上标记有地高辛，抗体识别地高辛进行显色。



【探针信息】

【组织固定】

组织类型	动物组织	植物组织	冰冻样本	细胞爬片	细胞
处理方式	室温固定 12h，石蜡包埋。	抽真空固定 1h，室温固定 12h，石蜡包埋。	15%蔗糖溶液中，4℃脱水 8 小时，换 30%蔗糖溶，液 4℃脱水 8 小时，OCT 包埋。	4°固定 2h 左右。	贴壁细胞用 PBS 清洗，加入多聚甲醛，将细胞刮下来，收集到离心管中，4℃固定 2h，PBS 清洗，琼脂糖包埋。
种类	mRNA	lncRNA	circRNA	miRNA	rRNA
处理方式	室温固定 12h	室温固定 12h（小于 300bp 固定 24h）	室温固定 12h	室温固定 12h	室温固定 12h（小于 300bp 固定 24h）

【储存与运输】

冰袋（wet ice）运输；-20℃长期保存,短期于 4℃保存，有效期 6 个月。

【使用方法】

1. 脱蜡至水

依次将切片放入二甲苯Ⅰ15 min-二甲苯Ⅱ15 min-二甲苯Ⅲ15 min-无水乙醇 10 min-90%酒精 10 min-80%酒精 10 min -70%酒精 10 min -纯水冲洗。

2. 酶修复

等待切片完全干燥后，用组画笔（推荐 HKR14P 原位杂交专用组画笔）画出大小合适的疏水圈，在原位杂交仪中或湿盒中水平放置切片，将 100ul 果胶酶纤维素酶混合液滴于组织上，37℃孵育 30min，纯水冲洗终止反应。甩干切片上的液体，将 100ul 蛋白酶 K 修复液(1X)滴于组织上，37℃孵育 30min，纯水冲洗终止反应。

3. 封闭

甩干切片上的液体，每张切片滴加 100ul 封闭液，37℃孵育 30min，洗涤 1 次，每次 5min。**洗涤步骤：将装入玻片的玻片架放入洗液缸，倒入洗液（样本区域须被浸没），置于摇床上 5min，转速为 60。**

4. 探针杂交

甩干切片上的液体，每张切片滴加 100ul 的探针，37℃孵育 3h 或过夜，注意保持湿度以防干片。洗涤 5 次，每次 5min。**洗涤步骤：将装入玻片的玻片架放入洗液缸，倒入洗液（样本区域须被浸没），置于摇床上 5min，转速为 60。**

5. 预放大杂交

甩干切片上的液体，每张切片滴加 100ul 的预放大探针，37℃孵育 3h 或过夜，注意保持湿度以防干片。洗涤 5 次，每次 5min。**洗涤步骤：将装入玻片的玻片架放入洗液缸，倒入洗液（样本区域须被浸没），置于摇床上 5min，转速为 60。**

6. 探针放大

甩干切片上的液体，每张切片滴加 100ul 的放大探针，37℃孵育 1.5h，注意保持湿度以防干片。洗涤 5 次，每次 5min。**洗涤步骤：将装入玻片的玻片架放入洗液缸，倒入洗液（样本区域须被浸没），置于摇床上 5min，转速为 60。**

7. AP-鼠抗地高辛

甩干切片上的液体，每张切片滴加 100ul 的 AP-鼠抗地高辛（1x），37℃湿盒孵育 40min；洗涤 5 次，每次 5min。**洗涤步骤：将装入玻片的玻片架放入洗液缸，倒入洗液（样本区域须被浸没），置于摇床上 5min，转速为 60。**

8. 显色

每张切片滴加 100ul 的显色液（1mL 去离子水加 50ul 显色液 A 和 50ul 显色液 B），室温孵育 20min。纯水冲洗，终止反应。自然风干后，滴加封片剂封片。

【注意事项】

1. 本产品仅作科研用途。

2. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

【示例图片】