

# PolyBranch HCR™ Rapid RNA FISH Kit(Plant)

**Catalog Number:HKR11Z**

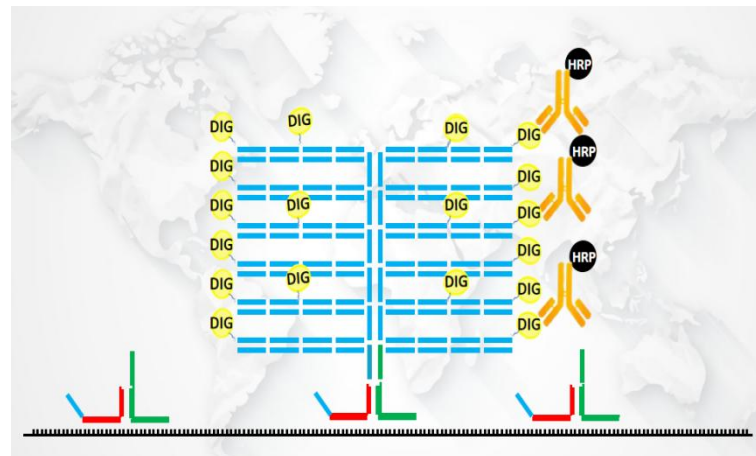
## 【Product Information】

| Product Name                                             | Catalog No | 20T   | Storage                         | Shelf Life |
|----------------------------------------------------------|------------|-------|---------------------------------|------------|
| Wash Buffer (powder, dissolve in 10L ddH <sub>2</sub> O) | HKR11-1    | 10L   | Short-term:4℃<br>Long-term:-20℃ | 12 months  |
| Proteinase K (100x)                                      | HKR11-2    | 20uL  |                                 |            |
| Pectinase/Cellulase Mixture                              | HKR11-3    | 2mL   |                                 |            |
| AP-Mouse Anti-Digoxin (100x)                             | HKR11-4    | 20uL  |                                 |            |
| NBT Chromogen Solution A (20x)                           | HKR11-5    | 100uL |                                 |            |
| NBT Chromogen Solution B (20x)                           | HKR11-6    | 100uL |                                 |            |
| Blocking Buffer                                          | HKR11-7    | 2mL   |                                 |            |
| Probe:                                                   | HKR11-8    | 2mL   |                                 |            |

## 【Product Description】

RNA rapid hybridization kit, suitable for in situ hybridization of RNA larger than 300bp, with simple and convenient operation and short experimental time. The reagent kit contains multiple pairs of trigger probes. When two probes bind to adjacent positions of the target sequence, one probe triggers the other probe to expose the amplified sequence. The amplified sequence triggers a branched HCR to form a large molecular nucleic acid aggregate, and each nucleic acid strand is labeled with digoxin. 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°C for 8h, then in 30% sucrose at 4°C for 8h, OCT embed. | Fix at 4°C for 2h. | scrape off cells, fix in 4% PFA at 4°C 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 °C for long-term or at 4 °C 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 °C 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 °C 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 °C 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 °C for 2 – 3 h (maintain humidity to prevent drying). Wash 5 times, 5 min each. **Wash steps: As described above.**

### 5. AP-Mouse Anti-Digoxin

Remove excess liquid from slides. Add 100 µL of AP-Mouse Anti-Digoxin (1X) per

slide and incubate at 37 °C in a humidified chamber for 40 min. Wash 5 times, 5 min each. **Wash steps: As described above.**

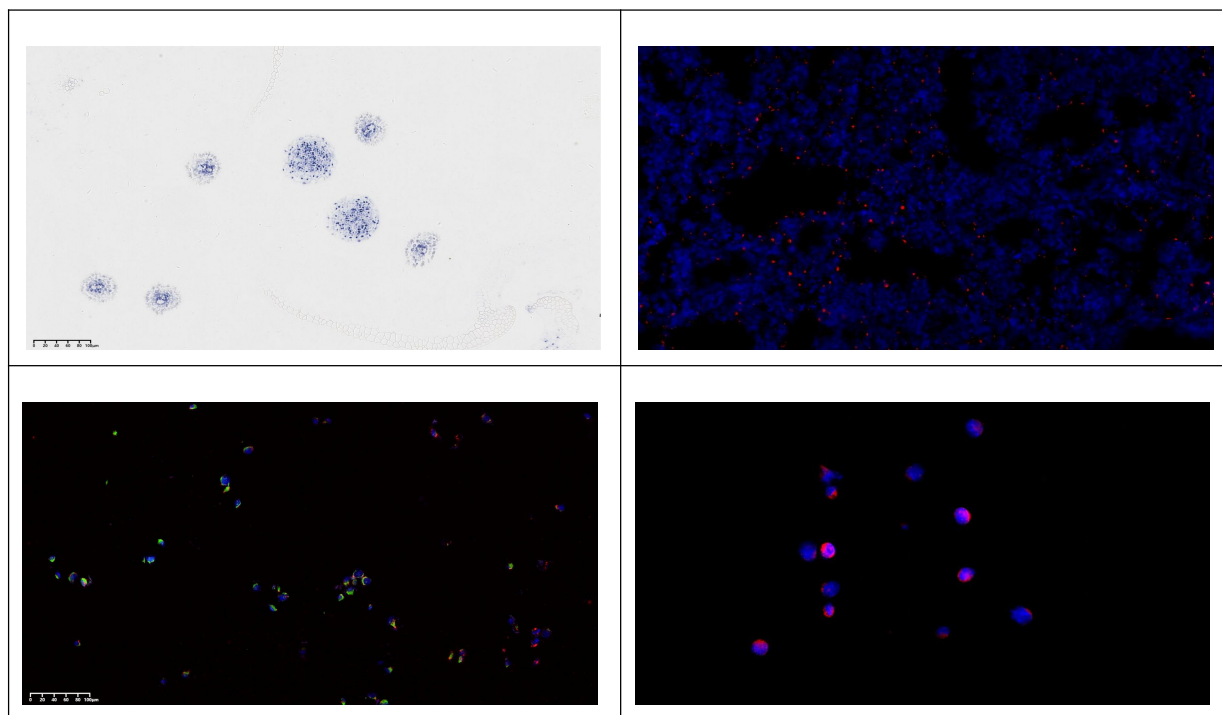
## 6. Chromogenic Reaction

Prepare the chromogenic solution by adding 50  $\mu$ L of NBT Chromogen Solution A and 50  $\mu$ L of NBT Chromogen Solution B to 1 mL of deionized water. Add 100  $\mu$ L 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 HCR™ Rapid RNA FISH Kit（植物）

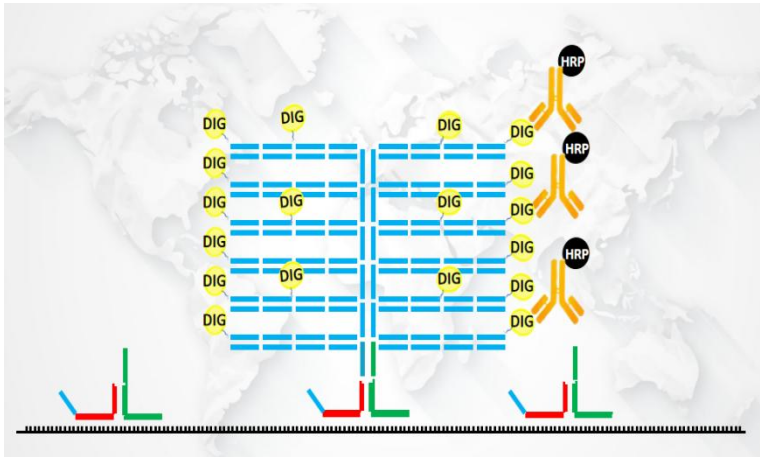
货号：HKR11Z

## 【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） | HKR11-1 | 10L   | 短期 4℃<br>长期 -20℃ | 12 个月 |
| 蛋白酶 K（100x）         | HKR11-2 | 20uL  |                  |       |
| 果胶酶纤维素酶混合液          | HKR11-3 | 2mL   |                  |       |
| AP-鼠抗地高辛（100x）      | HKR11-4 | 20uL  |                  |       |
| NBT 显色液 A（20x）      | HKR11-5 | 100uL |                  |       |
| NBT 显色液 B（20x）      | HKR11-6 | 100uL |                  |       |
| 封闭液                 | HKR11-7 | 2mL   |                  |       |
| 探针 NR_              | HKR11-8 | 2mL   |                  |       |

## 【产品简介】

RNA 快速杂交试剂盒，适用于大于 300bp 的 rna 原位杂交，操作简单便捷，实验时间短。探针中包含多对触发式探针，当两个探针结合在目标序列相邻的位置上时，一条探针触发另一条探针暴露出放大序列，放大序列触发树叉状 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<br>12h，石蜡包埋。 | 抽真空固定<br>1h，室温固定<br>12h，石蜡包埋。      | 15%蔗糖溶<br>液中，4℃脱<br>水 8 小时，换<br>30%蔗糖溶<br>液 4℃脱水 8<br>小时，OCT<br>包埋。 | 4° 固定 2h<br>左右。 | 贴壁细胞用<br>PBS 清洗，加<br>入多聚甲醛，<br>将细胞刮下<br>来，收集到离<br>心管中，4℃<br>固定 2h，PBS<br>清洗，琼脂糖<br>包埋。 |
| 种类   | mRNA              | lncRNA                             | circRNA                                                             | miRNA           | rRNA                                                                                   |
| 处理方式 | 室温固定<br>12h       | 室温固定<br>12h（小于<br>300bp 固定<br>24h） | 室温固定<br>12h                                                         | 室温固定<br>12h     | 室温固定<br>12h（小于<br>300bp 固定<br>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 I 15 min-二甲苯 II 15 min-二甲苯 III 15 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 2h-3h，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. 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。

## 6. 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 30min。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】

