HaoKebio™ circRNA FISH Kit(Plant)

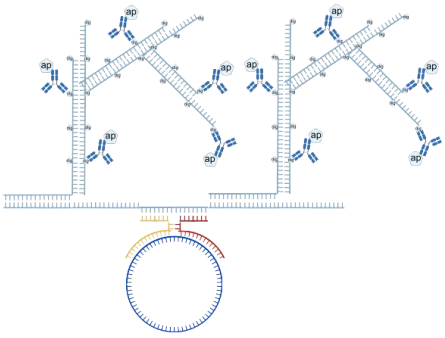
Catalog Number:HKR13Z-1

【Product Information】

Product Name	Catalog No	30T	Storage	Shelf Life
Wash Buffer (powder, dissolve in 10L ddH2O)	HKR13-1	10L	Short-term:4℃ Long-term:-20℃	12 months
AP-Mouse Anti-Digoxin (100x)	HKR13-2	30uL		
NBT Chromogen Solution A (20x)	HKR13-3	150uL		
NBT Chromogen Solution B (20x)	HKR13-4	150uL		
Blocking Buffer	HKR13-5	3mL		
Probe:	HKR13-6	3mL		
Pre-Amplification Probe	HKR13-7	3mL		
Amplification Probe	HKR13-8	3mL		

【Product Description】

The circRNA 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. Repair

Cell Samples:After the sections are completely dry, use a histology pen (recommended: HKR14P in situ hybridization-specific histology pen) to draw a hydrophobic circle of appropriate size. Place the slides horizontally in an in situ hybridization instrument or a humid chamber. Apply 100 μL of Proteinase K repair solution (1X) onto the tissue and incubate at 37°C for 10 minutes. Rinse with pure water to stop the reaction.

Frozen Sections:For cell samples, after the sections are completely dry, use a histology pen (recommended: HKR14P in situ hybridization-specific histology pen) to draw a hydrophobic circle of appropriate size. Place the slides horizontally in an in situ hybridization instrument or a humid chamber. Apply 100 μL of Proteinase K repair solution (1X) onto the tissue and incubate at 37°C for 20 minutes (for frozen sections). Rinse with pure water to stop the reaction.

Paraffin Sections:Place the slide rack containing the slides into the repair chamber and pour in 1X citrate buffer (pH 6.0) repair solution (ensure the sample area is fully submerged). Cover the chamber, seal it with tape, and place it in a microwave oven. Heat at medium power for 8 minutes, rest for 8 minutes, then heat at medium-low power for another 8 minutes. Allow the solution to cool naturally, then block with 3% hydrogen peroxide for 15 minutes.

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

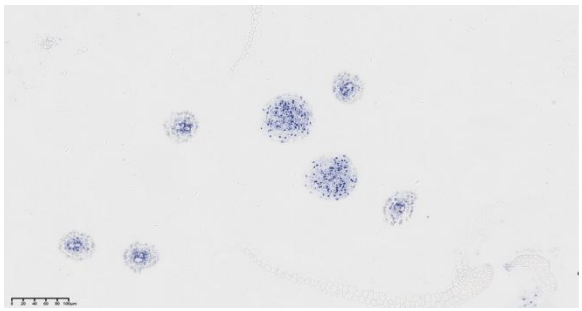
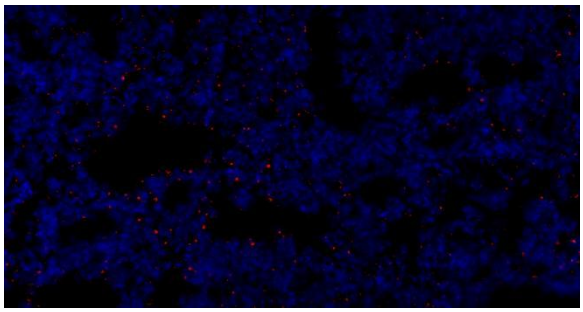
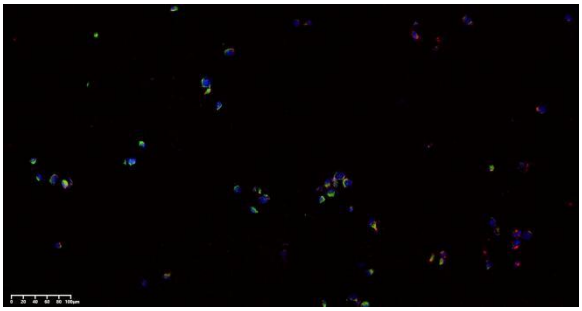
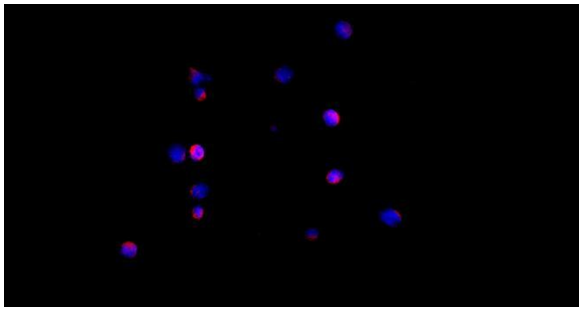
Prepare the chromogenic solution by adding 50 µL of NBT Chromogen Solution A and 50 µL of NBT Chromogen Solution B to 1 mL of deionized water. Add 100 µL of the mixture per slide and incubate at RT for 30 min. Rinse with distilled water to stop the reaction. Air-dry slides and mount with mounting medium.

【Precautions】

1. For research use only.

2. Wear lab coats and disposable gloves for safety.

【Example Images】

【Source of Reagents】

发表[中文论文]请标注:circRNA FISH Kit(Plant)(HKR13Z-1)由杭州浩克生物技术有限公司提供;

发表[英文论文]请标注:circRNA FISH Kit(Plant)(HKR13Z-1) were kindly provided by Hangzhou Haoke Biotechnology Co., Ltd.