

HaoKebio™ RNA FISH Kit(Plant)

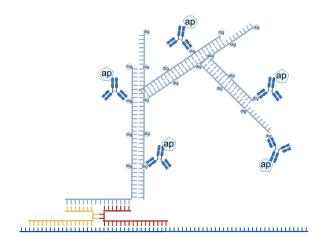
Catalog Number: HKR12Z

[Product Information]

Product Name	Catalog No	30T	Storage	Shelf Life
Wash Buffer (powder, dissolve in 10L ddH2O)	HKR12-1	10L		
AP-Mouse Anti-Digoxin (100x)	HKR12-2	30uL		
NBT Chromogen Solution A (20x)	HKR12-3	150uL	Short-term:4°C	12
NBT Chromogen Solution B (20x)	HKR12-4	150uL	Long-term:-20°C	months
Blocking Buffer	HKR12-5	3mL		
Probe:	HKR12-6	3mL		
Amplification Probe	HKR12-7	3mL		

[Product Description]

RNA hybridization kit, suitable for in situ hybridization of RNA larger than 300bp, with good specificity and high sensitivity. The reagent kit contains multiple pairs of combination probes. When two probes bind to adjacent positions of the target sequence, a small segment of the top sequence can trigger a branched HCR to form a large molecular 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	Cell Climb	Cells
			Samples	Slides	
Treatment	Fix at RT	Vacuum fix	Dehydrate in	Fix at 4℃	scrape off
	for 12h,	for 1h, RT fix	15% sucrose	for 2h.	cells,fix in
	paraffin	for 12h,	at 4℃ for		4% PFA at
	embed	paraffin	8h,then in		4°C for 2h,
		embed.	30% sucrose		wash with
			at 4℃ for		PBS, agarose
			8h, OCT		embed.
			embed.		
Туре	mRNA	lncRNA	circRNA	miRNA	rRNA
Treatment	Fix at RT for	Fix at RT for	Fix at RT for	Fix at RT for	Fix at RT for
	12h	12h (<300bp:	12h	12h	12h (<300bp:
		24h)			24h)

【Storage and Shipping】

Ship on wet ice; store at -20 $^{\circ}$ C for long-term or at 4 $^{\circ}$ C for short-term use. Shelf life: 6

months.

[Protocol]

1. Deparaffinization and Rehydration

Immerse slides sequentially in:Xylene I (15 min) \rightarrow Xylene II (15 min) \rightarrow Xylene III (15 min) \rightarrow 100% ethanol (10 min) \rightarrow 90% ethanol (10 min) \rightarrow 80% ethanol (10 min) \rightarrow 70% ethanol (10 min) \rightarrow 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°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 3 h or overnight (maintain humidity to prevent drying). Wash 5 times, 5 min each. Wash steps: As described above.

5. Probe Amplification

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

6. AP-Mouse Anti-Digoxin

Remove excess liquid from slides. Add 100 μ L of AP-Mouse Anti-Digoxin (1X) per slide and incubate at 37 $^{\circ}$ C in a humidified chamber for 40 min. Wash 5 times, 5 min each. Wash steps: As described above.

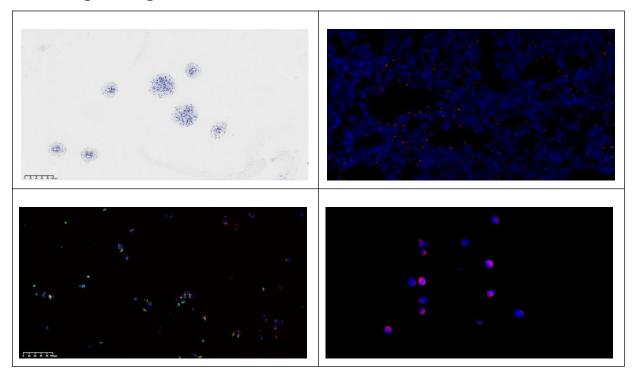
7. Chromogenic Reaction

Prepare the chromogenic solution by adding 50 μ L of NBT Chromogen Solution A and 50 μ L of NBT Chromogen Solution B to 1 mL of deionized water. Add 100 μ 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]



Source of Reagents

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