mutaFISH™ IFNGwt RNA Probes

Catalog Number FP0031

10 Assays

Version: 1.1

Intended for research use only

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Introduction

Intended Use

mutaFISH™ IFNGwt RNA Probes is designed to detect IFNG gene on single strand RNA in cells using *in situ* rolling-circle amplification technology.

General Information

Materials Supplied

Component	Qty.	Storage temp.
RT IFNG Primer	10 µL	-20℃
mutaFISH™ IFNGwt RNA Probe	10 µL	-20℃
Detection Probe-FITC	10 µL	-20℃

Reagent Required but Not Provided

- ✓ 100% ethanol.
- ✓ 85% ethanol: a solution of 85% ethanol
- ✓ 70% ethanol: a solution of 70% ethanol
- ✓ 3% paraformaldehyde

*mutaFISH™ RNA Accessory Kit (Catalog#: KA4915) contains reagents for cell *in situ* hybridization and CTC *in situ* hybridization. When frozen tissue and FFPE tissue is used with mutaFISH™ probe, additional DEPC-H2O, DEPC-PBS and PBS-T for immersing the slide should be prepared by the user.

Storage Instruction

Store at -20°C. Aliquot to avoid repeated freezing and thawing.

Note

We recommend mutaFISH™ RNA Accessory Kit (Catalog #: KA4915) which provides necessary reagents and enzymes for *in situ* reverse transcription, RNA digestion, mutaFISH™ hybridization, ligation and amplification prior to mutaFISH™.

Precautions

- General Precautions

✓ For research use only.
✓ Wear gloves and laboratory coats when handling immunodiagnostic materials.
✓ Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
✓ Wear gloves and eye and clothing protection when handling these reagents.
✓ The materials must not be pipetted by mouth.

- General Technical Hints

✓ Reagents with different lot numbers should not be mixed.
✓ Use thoroughly cleaned glassware.
✓ Use deionized (distilled) water, stored in clean containers.
✓ Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
✓ Dispose consumable materials and unused contents in accordance with applicable national regulatory requirements.

- Technical hints for Cell *In Situ* Hybridization

✓ Attached cells could be prepared by culturing on poly-L-lysine treated cover slide. For convenience, the cover slide with growth cells could be placed on a microscope slide before starting mutaFISH™ procedures.
✓ Suspension or attached cells could be adhered on poly-L-lysine treated microscope slide by Cytospin.
✓ Cell *In Situ* Hybridization protocol is suitable for 240 mm^2 cell area for each assay, user should make sure reagents applied could cover all of cell area.
✓ Use RNase free and DNase free tips for handling all regents
✓ Perform immunostaining prior to mutaFISH™ procedures. After immunostaining is completed, follow Cell *In Situ* Hybridization protocol below.

Protocol

Cell *In Situ* Hybridization

1. Fix cells with 100 µL of 2% paraformaldehyde for 15 min at RT.
2. Wash cells twice with 100 µL of DEPC-PBS.
3. **Fixation:** Pipette 100 µL of 3% paraformaldehyde into the slide and incubate the slide at RT for 30 minutes in humidity box.
4. **Wash step:** Pipette 100 µL of DEPC-PBS to the slide for 1 minute to remove the residual 3% paraformaldehyde.
5. Repeat the wash step.
6. **Dehydration:** Pipette 100 µL of 70%, 85% and 100% ethanol to the slide for 1 minute each.
7. **Permeabilization:** Wash the slide with 100 µL of PBS-T followed by 100 µL of permeabilization buffer at RT for 10 minutes.
8. **Wash step:** Wash the slide with 100 µL of PBS-T twice.
9. ***In Situ* RT:** The reaction contents are indicated in the **Table 1** below. Pipette 100 µL of total mixture to the slide and incubate the slide at 37°C for 3 hours in humidity box. .

Table 1.

Components of the reaction mixture	**Amount (µL)**
DEPC-H_2O	60.5
5X RT Buffer	20
BSA	1
dNTP Mix	5
RT IFNG Primer	1
RNase Inhibitor	2.5
RT Enzyme	10
Total reaction volume (µL)	**100**

10. **Wash step:** Wash the slide twice with 100 µL of PBS-T.
11. **Postfixation:** Pipette 100 µL of 3% paraformaldehyde to the slide and incubate the slide at RT for 30 minutes in humidity box.S
12. **Wash step:** Wash the slide twice with 100 µL of PBS-T.
13. **mutaFISH™ probe hybridization:** The reaction contents are indicated in the **Table 2** below. Pipette 100 µL of total mixture to the slide and incubate the slide at 37°C for 30 minutes, followed by at 45 °C for 45 min in humidity box.

Table 2.

Components of the reaction mixture	Amount (µL)
DEPC-H_2O	33.5
Formamide	20
10X Hybrid Enzyme Buffer	10
1M KCL	5
mutaFISH™ IFNGwt RNA Probe	1
RNase Inhibitor	2.5
RNase H	8
Hybrid Enzyme	20
Total reaction volume (µL)	**100**

14. **Wash step:** Wash the slide twice with 100 µL of PBS-T.
15. **Amplification:** The reaction contents are indicated in the **Table 3** below. Pipette 100 µL of total mixture to the slide and incubate the slide for 2 hours at 37°C in humidity box.

Table 3.

Components of the reaction mixture	Amount (µL)
DEPC-H_2O	61.5
50% Glycerol	10
10X DNA Polymerase Buffer	10
BSA	1
dNTP Mix	5
RNase Inhibitor	2.5
DNA Polymerase	10
Total reaction volume (µL)	**100**

16. **Wash step:** Wash the slide twice with 100 µL of PBS-T.
17. **Detection:** The reaction contents are indicated in the **Table 4** below. Pipette 100 µL of total mixture to the slide and incubate the slide for 15 minutes at 37°C in humidity box.

Table 4.

Components of the reaction mixture	**Amount (µL)**
Detection Buffer	99
Detection Probe-FITC	1
Total reaction volume (µL)	**100**

18. **Wash step:** Wash the slide twice with 100 µL of PBS-T.
19. **Dehydration:** Pipette 100 µL of 70%, 85% and 100% ethanol to the slide for 30 seconds each.
20. **Counter Stain:** Apply 100 µL of 300nM DAPI (Add 1 ul of 100 µM DAPI and DEPC-PBS to 333 µL) to the slide at RT for 3 minutes.
21. Wash the slide with 100 µL of DEPC-PBS twice. The slide is now ready for examine under fluorescence microscope.

CTC *In Situ* Hybridization

Bring Components and mixtures prepared below to room temperature before applying to the chip

1. Wash cells twice with 100 µL of DEPC-PBS.
2. **Fixation:** Pipette 100 µL of 3% paraformaldehyde into the chip and incubate the chip at RT for 30 minutes in humidity box.
3. **Wash step:** Pipette 100 µL of DEPC-PBS into the chip for 1 minute to remove the residual 3% paraformaldehyde.
4. Repeat the wash step.
5. **Dehydration:** Pipette 100 µL of 70%, 85% and 100% ethanol into the chip for 1 minute each.
6. **Permeabilization:** Wash the chip with 100 µL of PBS-T followed by 100 µL of permeabilization buffer at RT for 10 minutes.
7. **Wash step:** Wash the chip with 100 µL of PBS-T twice.
8. **In Situ RT:** The reaction contents are indicated in the **Table 5** below. Pipette 100 µL of total mixture into the chip and incubate the chip at 37°C for 3 hours in humidity box. Make sure the inlet and outlet of the chip are consistently covered with DEPC-PBS throughout incubation.

Table 5.

Components of the reaction mixture	**Amount (µL)**
DEPC-H_2O	60.5
5X RT Buffer	20
BSA	1
dNTP Mix	5
RT IFNG Primer	1
RNase Inhibitor	2.5
RT Enzyme	10
Total reaction volume (µL)	**100**

9. **Wash step:** Wash the chip twice with 100 µL of PBS-T.
10. **Postfixation:** Pipette 100 µL of 3% paraformaldehyde into the chip and incubate the chip at RT for 30 minutes in humidity box.
11. **Wash step:** Wash the chip twice with 100 µL of PBS-T.
12. **mutaFISH™ probe hybridization:** The reaction contents are indicated in the **Table 6** below. Pipette 100 µL of total mixture into the chip and incubate the chip at 37°C for 30 minutes, followed by at 45 °C for 45 min in humidity box. Make sure the inlet and outlet of the chip are consistently covered with DEPC-PBS throughout incubation.

Table 6.

Components of the reaction mixture	Amount (µL)
DEPC-H_2O	33.5
Formamide	20
10X Hybrid Enzyme Buffer	10
1M KCL	5
mutaFISH™ IFNGwt RNA Probe	1
RNase Inhibitor	2.5
RNase H	8
Hybrid Enzyme	20
Total reaction volume (µL)	**100**

13. **Wash step:** Wash the chip twice with 100 µL of PBS-T.
14. **Amplification:** The reaction contents are indicated in the **Table 7** below. Pipette 100 µL of total mixture into the chip and incubate the chip for 2 hours at 37°C in humidity box. Make sure the inlet and outlet of the chip are consistently covered with DEPC-PBS throughout incubation.

Table 7.

Components of the reaction mixture	Amount (µL)
DEPC-H_2O	61.5
50% Glycerol	10
10X DNA Polymerase Buffer	10
BSA	1
dNTP Mix	5
RNase Inhibitor	2.5
DNA Polymerase	10
Total reaction volume (µL)	**100**

15. **Wash step:** Wash the chip twice with 100 µL of PBS-T.
16. **Detection:** The reaction contents are indicated in the **Table 8** below. Pipette 100 µL of total mixture into the chip and incubate the chip for 15 minutes at 37°C in humidity box. Make sure the inlet and outlet of the chip are consistently covered with DEPC-PBS throughout incubation.

Table 8.

Components of the reaction mixture	Amount (μL)
Detection Buffer	99
Detection Probe-FITC	1
Total reaction volume (μL)	**100**

17. **Wash step:** Wash the chip twice with 100 μL of PBS-T.
18. **Dehydration:** Pipette 100 μL of 70%, 85% and 100% ethanol into the chip for 30 seconds each.
19. **Counter Stain:** Apply 100 μL of 300nM DAPI (Add 1 ul of 100 μM DAPI and DEPC-PBS to 333 μL) at RT for 3 minutes.
20. Wash the chip with 100 μL of DEPC-PBS twice. The chip is now ready for examine under fluorescence microscope.

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