

## Preparation of FISH probe

1. The following FISH probes are ready-to-use, no need of any preparation.
  - a. Gene FISH Probe (Cat # FGxxxx)
  - b. Split FISH Probe (Cat # FSxxxx)
  - c. Translocation FISH Probe (Cat # FTxxxx)
  - d. Prenatal FISH Probe (Cat # FMxxxx)
  - e. Made to Order FISH Probe (Ca # FAxxxx)
  
2. Chromosome FISH Probe (Cat # FCxxxx) and Subtelomere FISH Probe (Cat # FExxxx) are provided in 5x concentrated format, they should be either:
  - a. Diluted to 1x with FISH Hybridization Buffer (Cat # [U0028](#) or [U0029](#)) before use,  
OR
  - b. Mixed with same category of FISH Probes (up to 5 different probes) to use, for example:
 

**Combine 2 different probes:**

 1 volume of probe 1 (2 uL) + 1 volume of probe 2 (2 uL)  
 + 3 volume of FISH Hybridization Buffer (6 uL)

**Combine 3 different probes:**

 1 volume of probe 1 (2 uL) + 1 volume of probe 2 (2 uL)  
 + 1 volume of probe 3 (2 uL) + 2 volume of FISH Hybridization Buffer (4 uL)

## Recommended filter set

The table below is a recommendation of filter set use:

| Fluorophore                         | Brand   | Recommended filter set |
|-------------------------------------|---------|------------------------|
| Single fluorophore:                 |         |                        |
| <b>FITC</b> (EX. 495; EM. 520)      | Semrock | SpGr-B                 |
| <b>Texas Red</b> (EX. 593; EM. 612) | Semrock | SpRed-B                |
| <b>DEAC</b> (EX. 426; EM. 480)      | Semrock | SpAqua-C               |
| <b>R6G</b> (EX. 525; EM. 550)       | Semrock | SpGold-B               |
| <b>Cy5</b> (EX. 650; EM. 668)       | Semrock | CY5-4040B or CY5-4040C |
| Multiple fluorophores:              |         |                        |
| <b>FITC, Texas Red &amp; DAPI</b>   | Semrock | DA/SpGr/SpRed-A        |

Note: EX. = excitation wavelength; EM. = emission wavelength

## Protocol selection

Please follow an appropriate protocol below depend on the sample use, these samples include **Paraffin embedded tissue (or FFPE), Frozen tissue and Metaphase spreads.**

For **Paraffin embedded tissue**, we recommended **FFPE FISH PreTreatment Kit 1**(Catalog #: [KA2375](#) or [KA2691](#) for the pretreatment of Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections.

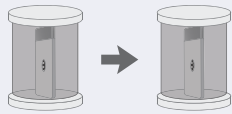
## Paraffin embedded tissue

### 1. Deparaffinized



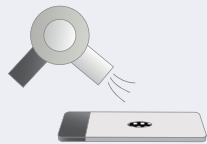
Xylene 5 min×3  
Room temperature

### 2. Dehydrate



100% EtOH 5 min×2  
Room temperature

### 3. Air dry



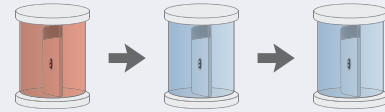
### 4. Pre-treatment



Paraffin  
Pretreatment  
Solution  
95°C 30 min

Wash buffer  
(2×SSC)  
5 min×2

### 5. Protease treatment

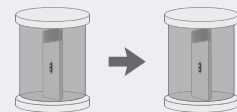


Protease Solution  
37°C 10~20min  
Wash buffer  
(2×SSC) 5 min×2

\*Protease Solution  
Add 500µl protease in 50ml  
protease buffer

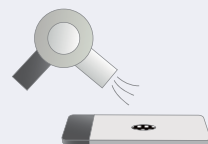
\*Protease preservation  
One month : 4°C  
Over one month : -20°C

### 6. Dehydrate (Room temperature)



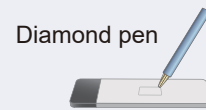
70% EtOH 1 min  
100% EtOH 1 min

### 7. Air dry

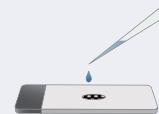


## FISH protocol

### 1. Mark hybridizing area



### 2. Apply 10µl FISH probe for 22mm x 22mm area



### 3. Cover with cover glass and seal with rubber cement



### 4. Denature



75°C 5 min

## Hybridization

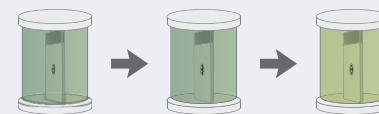
### 1. Incubation



Humidified box  
37°C 16 ~ 72 hrs

## Wash procedure

Remove rubber cement  
Slide into 2X SSC and remove  
cover glass



2X SSC Room temp.  
5 min

2X SSC /0.3% NP-40  
73~75°C  
1-2min

2X SSC Room temp.  
1 min

## Counter stain

### 1. Apply 10µl DAPI Solution to target area

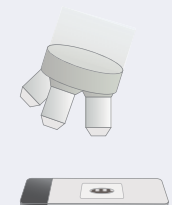


\*DAPI Paraffin embedded  
tissue 1500ng/ml

### 2. Put on cover glass Seal with manicure



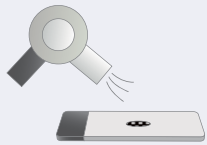
## Examine



## Frozen tissue

1. Frozen tumour tissue

2. Air dry



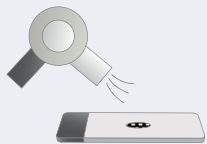
Positive charged slides

3. Fix and Dehydrated

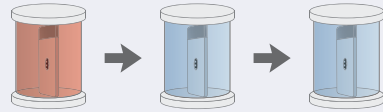


95%EtOH  
20min

4. Air dry



5. Protease treatment

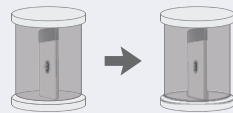


Protease Solution 37°C 10~20min  
Wash buffer (2×SSC) 5 min×2

\*Protease Solution  
Add 50µl protease in protease buffer

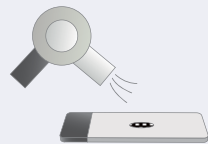
\*Protease preservation  
One month : 4°C  
Over one month : -20°C

6. Dehydrate (Room temperature)



70% EtOH 1 min  
100% EtOH 1 min

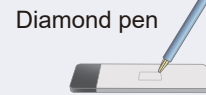
7. Air dry



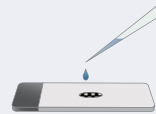
touch preparations of unfixed tumour tissue/cell smears/cytospins of cultured or blood cells are possible

## FISH protocol

1. Mark hybridizing area



2. Apply 10µl FISH probe for 22mm x 22mm area



3. Cover with cover glass and seal with rubber cement



4. Denature



75°C 5 min

## Hybridization

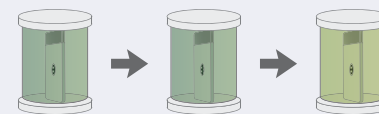
1. Incubation



Humidified box  
37°C 16 ~ 72 hrs

## Wash procedure

Remove rubber cement  
Slide into 2X SSC and remove cover glass



2X SSC Room temp. 5 min  
2X SSC /0.3% NP-40 73~75°C 1-2min  
2X SSC Room temp. 1 min

## Counter stain

1. Apply 10µl DAPI Solution to target area

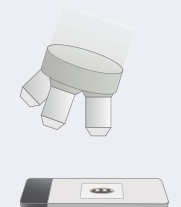


\*DAPI  
Frozen tumour tissue  
150ng/ml

2. Put on cover glass  
Seal with manicure



## Examine



## Metaphase spreads

### 1. Ageing

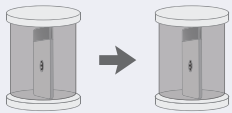


37°C 30min

Ageing solution  
(2XSSC/0.1% NP-40:PH7~8)

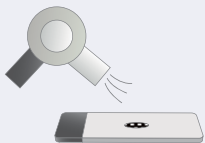
|         |      |
|---------|------|
| 20X SSC | 5ml  |
| NP-40   | 50µl |
| DDW     | 45ml |

### 2. Dehydrate (Room temperature)



70% EtOH 1min      100% EtOH 1min

### 3. Air dry



## FISH protocol

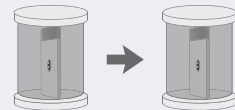
### 1. Slide preparation



73~75°C 5min  
Denaturant Solution  
(2XSSC/70%formamide : PH7~8)

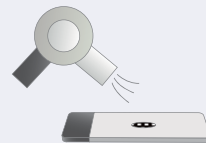
|               |      |
|---------------|------|
| 100%formamide | 35ml |
| 20XSSC        | 5ml  |
| DDW           | 10ml |

### 2. Dehydrate (Room temperature)



70% EtOH 1min      100% EtOH 1min

### 3. Air dry



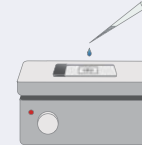
## Probe preparation



10µl  
73~75°C 5min

## Hybridization

### 1. Apply 10µl FISH probe for 22mm x 22mm area



45~50°C

### 2. Cover with cover glass Seal with rubber cement



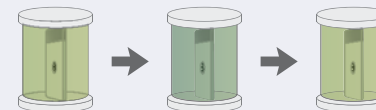
### 3. Incubation



Humidified box  
37°C 16 ~ 72 hrs

## Wash procedure

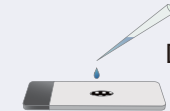
Remove rubber cement  
Slide into 2X SSC and remove  
cover glass



2X SSC Room temp. 5 min      0.4X SSC /0.3% NP-40 73~75°C 1-2min      2X SSC Room temp. 1 min

## Counter stain

### 1. Apply 10µl DAPI Solution to target area



DAPI 10µl

\*DAPI  
Metaphase spreads  
150ng/ml

### 2. Put on cover glass Seal with manicure



## Examine

