

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:		
FITC (EX. 495; EM. 520)	Semrock	SpGr-B
Texas Red (EX. 593; EM. 612)	Semrock	SpRed-B
DEAC (EX. 426; EM. 480)	Semrock	SpAqua-C
R6G (EX. 525; EM. 550)	Semrock	SpGold-B
Cy5 (EX. 650; EM. 668)	Semrock	CY5-4040B or CY5-4040C
Multiple fluorophores:		
FITC, Texas Red & DAPI	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.

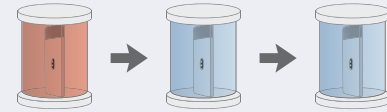
Paraffin embedded tissue

1. Deparaffinized



Xylene 5 min×3
Room temperature

5. Protease treatment

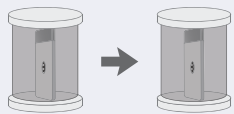


Protease Solution 37°C 10~20min
Wash buffer (2×SSC) 5 min×2

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

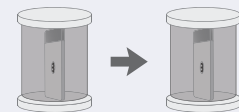
*Protease preservation
One month : 4°C
Over one month : -20°C

2. Dehydrate



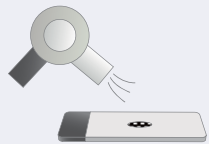
100% EtOH 5 min×2
Room temperature

6. Dehydrate (Room temperature)



70% EtOH 1 min
100% EtOH 1 min

3. Air dry

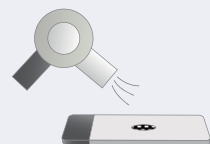


4. Pre-treatment



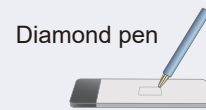
Paraffin Pretreatment Solution 95°C 30 min
Wash buffer (2×SSC) 5 min×2

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

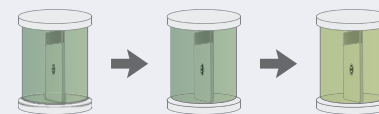
1. Incubation



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

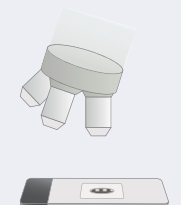


*DAPI Paraffin embedded tissue 1500ng/ml

2. Put on cover glass Seal with manicure



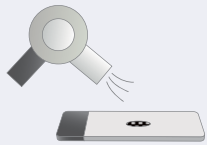
Examine



Frozen tissue

1. Frozen tumour tissue

2. Air dry



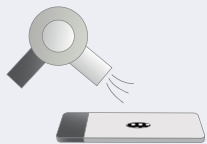
Positive charged slides

3. Fix and Dehydrated

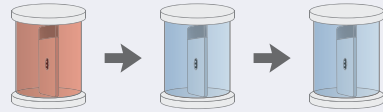


95%EtOH
20min

4. Air dry



5. Protease treatment

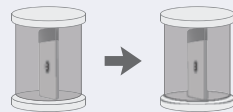


Protease Solution 37°C 10~20min
Wash buffer (2×SSC) 5 min×2

*Protease Solution
Add 50µl protease in protease buffer

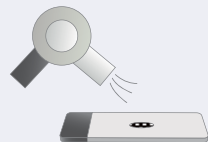
*Protease preservation
One month : 4°C
Over one month : -20°C

6. Dehydrate (Room temperature)



70% EtOH 1 min
100% EtOH 1 min

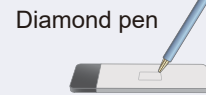
7. Air dry



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

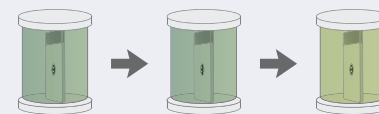
1. Incubation



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

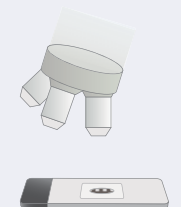


*DAPI
Frozen tumour tissue
150ng/ml

2. Put on cover glass
Seal with manicure



Examine



Metaphase spreads

1. Ageing

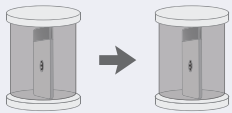


37°C 30min

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

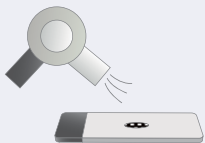
20X SSC	5ml
NP-40	50µl
DDW	45ml

2. Dehydrate (Room temperature)



70% EtOH 1min 100% EtOH 1min

3. Air dry



FISH protocol

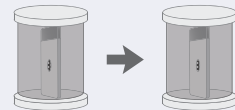
1. Slide preparation



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

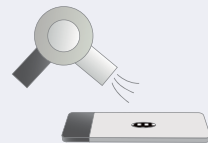
100%formamide	35ml
20XSSC	5ml
DDW	10ml

2. Dehydrate (Room temperature)



70% EtOH 1min 100% EtOH 1min

3. Air dry



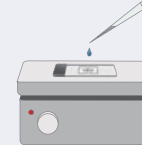
Probe preparation



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



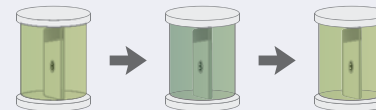
3. Incubation



Humidified box
37°C 16 ~ 72 hrs

Wash procedure

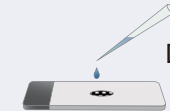
Remove rubber cement
Slide into 2X SSC and remove
cover glass



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

