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.**

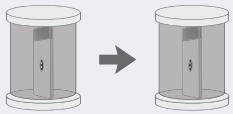
For **Paraffin embedded tissue**, we recommended **FFPE FISH PreTreatment Kit 1**(Catalog #: KA2375 or KA2691 for the pretreatment of Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections.

Paraffin embedded tissue

1. Deparaffinized

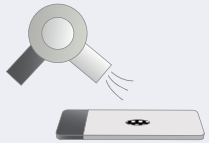
Xylene 5 min×3
Room temperature

2. Dehydrate



100% EtOH 5 min×2
Room temperature

3. Air dry



4. Pre-treatment

Paraffin Pretreatment Solution
95℃ 30 min

Wash buffer (2×SSC)
5 min×2

5. Protease treatment

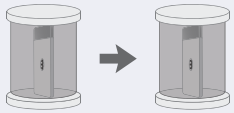
Protease Solution
37℃ 10～20min

Wash buffer
(2×SSC) 5 min×2

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

*Protease preservation
One month：4℃
Over one month：-20℃

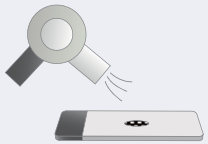
6. Dehydrate (Room temperature)



70% EtOH
1 min

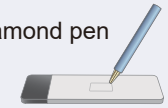
100% EtOH
1 min

7. Air dry

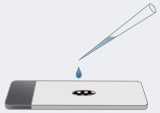


FISH protocol

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℃ 5 min

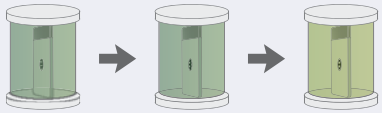
Hybridization

1. Incubation

Humidified box
37℃ 16 ~ 72 hrs

Wash procedure

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



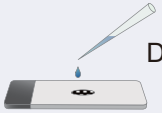
2X SSC
Room temp.
5 min

2X SSC
/0.3% NP-40
73~75℃
1-2min

2X SSC
Room temp.
1 min

Counter stain

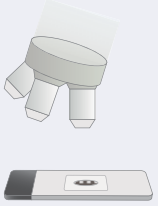
1. Apply 10µl DAPI Solution to target area



*DAPI Paraffin embedded tissue 1500ng/ml

2. Put on cover glass
Seal with manicure

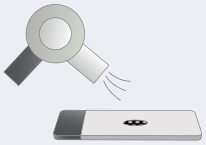
Examine



Frozen tissue

1. Frozen tumour tissue

2. Air dry

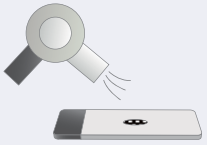


Positive charged slides

3. Fix and Dehydrated

95%EtOH
20min

4. Air dry



5. Protease treatment

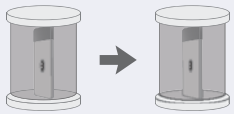
Protease Solution
37℃ 10～20min

Wash buffer
(2×SSC) 5 min×2

*Protease Solution
Add 50μl protease in protease buffer

*Protease preservation
One month：4℃
Over one month：-20℃

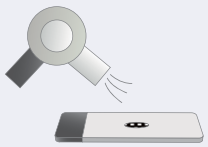
6. Dehydrate (Room temperature)



70% EtOH
1 min

100% EtOH
1 min

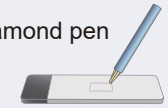
7. Air dry



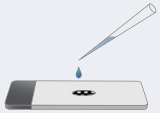
touch preparations of unfixed tumourtissue/cell smears/cytospins of cultured or blood cells are possible

FISH protocol

1. Mark hybridizing area



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



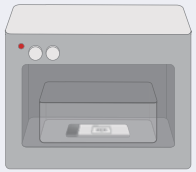
3. Cover with cover glass and seal with rubber cement

4. Denature

75℃ 5 min

Hybridization

1. Incubation



Humidified box
37℃ 16 ~ 72 hrs

Wash procedure

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

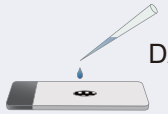
2X SSC
Room temp.
5 min

2X SSC
/0.3% NP-40
73~75℃
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

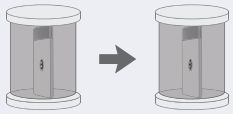
1. Ageing

37℃ 30min

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

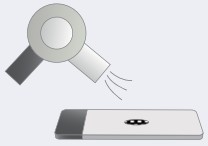
20X SSC	5ml
NP-40	50μl
DDW	45ml

2. Dehydrate
(Room temperature)



70% EtOH 1min
100% EtOH 1min

3. Air dry



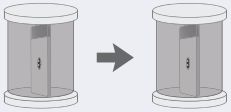
FISH protocol

1. Slide preparation

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

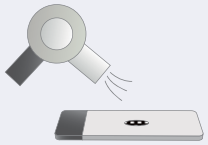
100%formamide	35ml
20XSSC	5ml
DDW	10ml

2. Dehydrate
(Room temperature)



70% EtOH 1min
100% EtOH 1min

3. Air dry



Probe preparation

10μl
73~75℃ 5min

Hybridization

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

45~50℃

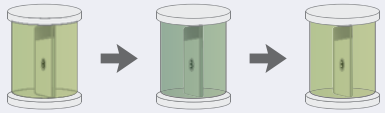
2. Cover with cover glass
Seal with rubber cement

3. Incubation

Humidified box
37℃ 16 ~ 72 hrs

Wash procedure

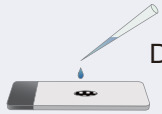
Remove rubber cement
Slide into 2X SSC and remove
cover glass



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

Counter stain

1. Apply 10μl DAPI
Solution to target area

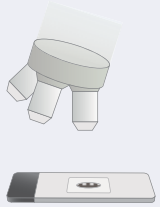


DAPI 10μl

*DAPI
Metaphase spreads
150ng/ml

2. Put on cover glass
Seal with manicure

Examine



(Ver. 2.0)

GSP Lab., Inc.