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. 426; EM. 480)	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
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**.

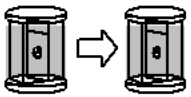
For **Paraffin embedded tissue**, we recommended **FFPE FISH PreTreatment Kit 1**(Catalog #: KA2375) for the pretreatment of Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections.

Paraffin embedded tissue

1. Deparaffinized

Xylene 5min×3
Room temperature

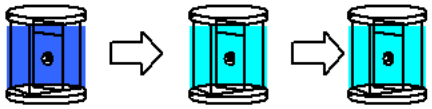
2. Dehydrate



100%EtOH 5min×2
Room temperature

3. Air dry

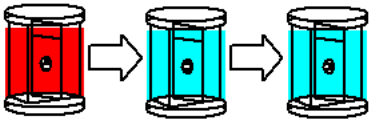
4. Pre-treatment



Paraffin Pretreatment Solution
95℃ 30min

Wash buffer (2×SSC)
5min × 2

5. Protease treatment

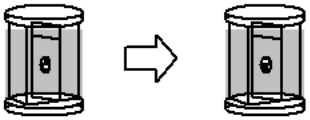


Protease Solution
37℃ 10～20min

Wash buffer (2×SSC)
5min × 2

☆Protease Solution
Add 500μ l protease in 50ml protease buffer
☆Protease preservation
One month : 4℃
Over one month : -20℃

6. Dehydrate
(Room temperature)



70% EtOH
1min

100% EtOH
1min

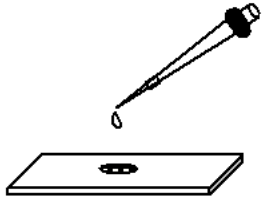
7. Air dry

FISH protocol

1. Mark hybridizing area

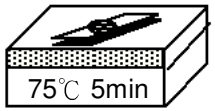
Diamond pen

2. Apply 10μl FISH probe
(22mm x 22mm area)



3. Cover with cover glass
Seal with rubber cement

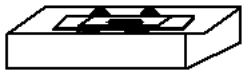
4. Denature



75℃ 5min

Hybridization

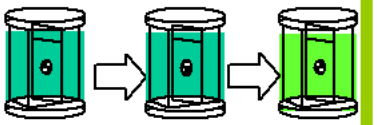
1. Incubation



Humidified box
37℃ 16 ~ 72 hrs

Wash procedure

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



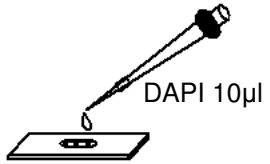
2X SSC
Room temp.
5min

2X SSC
/0.3% NP-40
73～75℃
1-2min

2X SSC
Room temp.
1min

Counter stain

1. Apply 10μl DAPI Solution to target area

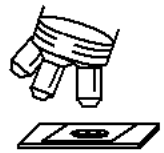


DAPI 10μl

☆DAPI
Paraffin embedded tissue
1500ng/ml

2. Put on cover glass
Seal with manicure

Examine



Frozen tissue

1. Frozen tumour tissue

2. Air dry

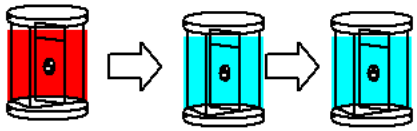
Positive charged slides

3. Fix and Dehydrated

95%EtOH
20min

4. Air dry

5. Protease treatment

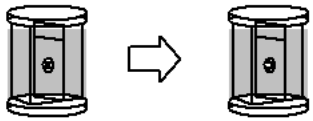


Protease Solution
37℃ 10～20min

Wash buffer
(2xSSC)
5min x 2

☆Protease Solution
Add 50μ l protease in protease buffer
☆Protease preservation
One month : 4℃
Over one month : -20℃

6. Dehydrate
(Room temperature)



70% EtOH
1min

100% EtOH
1min

7. Air dry

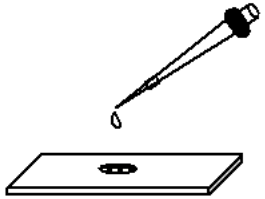
touch preparations of unfixed tumourtissue/cell smears/cytospins of culturedor blood cells are possible

FISH protocol

1. Mark hybridizing area

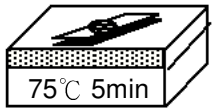
Diamond pen

2. Apply 10μl FISH probe
(22mm x 22mm area)



3. Cover with cover glass
Seal with rubber cement

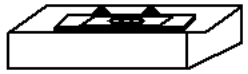
4. Denature



75℃ 5min

Hybridization

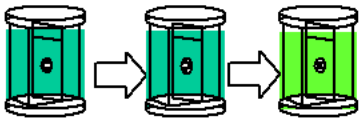
1. Incubation



Humidified box
37℃ 16 ~ 72 hrs

Wash procedure

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



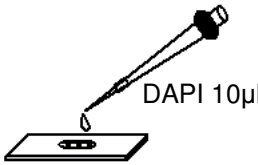
2X SSC
Room temp.
5min

2X SSC
/0.3% NP-40
73～75℃
1-2min

2X SSC
Room temp.
1min

Counter stain

1. Apply 10μl DAPI Solution to target area

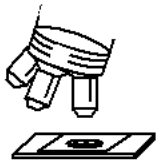


DAPI 10μl

☆DAPI
Frozen tumour tissue
150ng/ml

2. Put on cover glass
Seal with manicure

Examine



Metaphase spreads

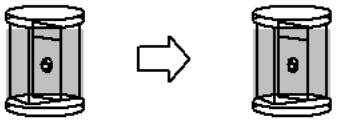
1. Ageing

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

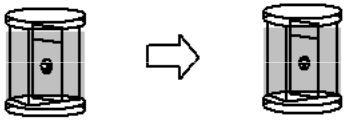
1. Slide preparation

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

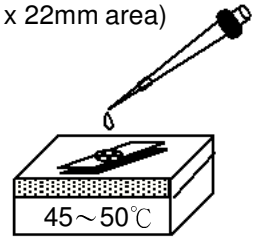
3. Air dry

Probe preparation

10μl
73～75℃ 5min

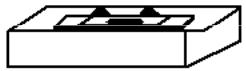
Hybridization

1. Apply 10μl FISH probe
(22mm x 22mm area)



2. Cover with cover glass
Seal with rubber cement

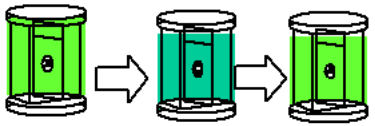
3. Incubation



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.
5min	73～75℃	1min
	1-2min	

Counter stain

1. Apply 10μl DAPI
Solution to target area

☆DAPI
Metaphase spreads
150ng/ml

2. Put on cover glass
Seal with manicure

Examine