Document Number: **SASoM/METHOD/145.v1**

Title: **Fluorescence In-situ hybridisation (FISH) in FFPE sections.**

Version: **v1**

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SOP History		
Number	Date	Reason for Change
v1	04/11/2021	Original

1.0 Purpose –

This SOP describes the current procedures for performing Fluorescence In-situ hybridisation (FISH) in FFPE sections in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to all staff performing Fluorescence In-situ hybridisation (FISH) in FFPE sections in Laboratory 248/249 at the St Andrews School of Medicine (SASoM).

3.0 Responsibilities –

All staff performing Fluorescence In-situ hybridisation (FISH) in FFPE sections in this manner are responsible for ensuring that the methods are followed in accordance with this SOP. All staff must have read and signed the relevant risk assessment documents before performing this procedure.

4.0 Procedure –

Fluorescence in-situ hybridisation (FISH) is a method whereby specific nucleic acid sequences can be detected in tissue sections using fluorophore-conjugated probes and viewed by fluorescence microscopy. It can be used to detect chromosome rearrangements and DNA copy number changes in a range of pathological conditions. This technique is a direct FISH assay suitable for detection of chromosome rearrangements in formalin-fixed paraffin-embedded sections for the diagnosis of certain lung and soft tissue tumours, and for detection of HER2 gene amplification in breast/gastric carcinoma. It has been validated for the probes listed below; others may be requested for research purposes only.

Probe available	Location	Associated Conditions
Dako pharmDx HER2	17q12	Breast/gastric carcinoma
Vysis LSI ALK	2p23	NSCLC lung cancer
Vysis LSI DDIT3 (formerly CHOP)	12q13	Myxoid liposarcoma
Vysis LSI EWSR1	22q12	Ewing's sarcoma
Vysis LSI FOXO1	13q14	Alveolar rhabdomyosarcoma
Vysis LSI FUS	16p11	Myxoid/fibromyxoid sarcoma
Vysis LSI MDM2	12q15	Soft tissue sarcoma
Vysis LSI SS18 (formerly SYT)	18q11.2	Synovial sarcoma
Abnova		Trib1
Abnova		cMYC

Material Requirements

The pre-treatment and hybridisation procedures described in this protocol are optimised for formalin-fixed paraffin-embedded tissue sections cut at a thickness of 4µm. Sections should be floated onto electrostatically charged slides to prevent lifting during harsh pre-treatment.

- **Solutions preparation**

Reagents from the Agilent Histology FISH Accessory Kit (K579911-2) are used for all pre-treatments. Stock reagents are stored at -20ºC until opened and 5ºC thereafter. Probes are aliquotted on opening (~5 tests per aliquot) and stored at -20ºC.

1. FISH Wash Buffer - Add 50ml of 20x Wash Buffer concentrate (K579911-2 Vial 6) to 950ml distilled water
2. Pre-Treatment Solution - Add 5ml of 20x Pre-Treatment Solution concentrate (K579911-2 Vial 1) to 95ml distilled water
3. Stringent Wash Buffer - Add 5ml of 20x Stringent Wash Buffer concentrate (K579911-2, Vial 4) to 95ml distilled water

- **Pre-treatment**

1. Switch on water bath and set to 95°C
2. Fill a weighted plastic Coplin jar with Pre-Treament Solution (K579911-2 **vial 1**) and place into the waterbath to pre-heat for 1 hour
3. Deparaffinise sections with fresh xylene for 3 x 5min
4. Hydrate sections through descending grades of alcohols (100%, 100%, 80% and 50%) to water then transfer to hot Pre-Treatment Solution in the waterbath. Gloves ***must*** be worn to prevent scalding.
5. Incubate for *exactly* **30min**
6. Remove Coplin jar from water bath and take off lid. Gloves ***must*** be worn. Allow to cool at room temperature for exactly 15min
7. Discard the Pre-Treatment Solution and transfer all slides to diluted 2xSSC buffer at room temperature 5min
8. Replace with fresh 2xSSC buffer and incubate for 5min
9. Tap off excess buffer and carefully wipe around the sections
10. Lay slides flat in a black wet chamber and add a few drops of cold Pepsin (K579911-2 Vial 2A) to cover the sections. ***Keep Pepsin in fridge until immediately before use and replace straight after use***
11. Incubate sections appropriately as follows, or shorter/longer where non-standard fixation requires:

Probe	Time	Temperature
Alk	20min	Room temp
Sarcoma	20min	Room temp
Her2	12min	Room temp
cMYC	6min	37deg
Trib1	6min	37deg

12. Tap off excess Pepsin on paper towel and place sections in PBST Wash Buffer for 3min
13. Replace with fresh PBST Wash Buffer and incubate for a further 3min
14. Dehydrate in fresh 70%, 85%, and 96% alcohol (2min each)
15. Air-dry sections

- **Denaturation and Hybridisation**

16. Apply the FISH probe:

    a) These probes are ***ready to use***. Select a coverslip large enough to cover the section and, under subdued lighting, apply the appropriate volume of probe as follows:

Coverslip	Probe Volume
10mm dia.	2µl
12 x 12mm	3µl
15 x 15mm	5µl
18 x 18mm	7µl
22 x 22mm	10µl
22 x 26mm	12µl
22 x 32mm	15µl
22 x 40mm	18µl
22 x 50mm	23µl

17. Gently press the section onto the coverslip, ensuring no air bubbles are trapped
18. Apply Coverslip Sealant around the periphery of the coverslip, making sure an adequate seal is formed. Allow to dry
19. Set up the HybEZ hybridiser (SASoM/EQUIP/076):
    a) Switch on the hybridiser and set the temperature at 72°C
    b) Moisten Humidity Control Strips (Agilent S245230-2) with distilled water and put into the bottom of the tray.
    c) Place the sealed slides into the HybEZ slide rack (maximum 20) and put the slide rack into the tray and close the lid of the tray.
    d) Insert tray into the hybridser (refer to SASoM/EQUIP/076) and incubate for 5min for denaturing process.
    e) Adjust the temperature to 37°C and leave the to run for 72 hours

- **Post-Hybridisation**

20. Fill two plastic Coplin jars, one weighted, with Stringent Wash Buffer
21. Place the weighted Coplin jar into the waterbath and pre-heat to 65°C for 1hr, leaving the other jar at room temperature
22. Take slides out of the Hybridiser, remove the coverslips and sealant and place slides in the jar of room temperature Stringent Wash Buffer

23. As soon as all coverslips have been removed, transfer the slides to the hot Stringent Wash Buffer in the waterbath. Gloves ***must*** be worn to prevent scalding
24. Incubate slides for *exactly* **10min**
25. Meanwhile, switch off the hybridiser and place the used Humidity Control Strips into a measuring cylinder of distilled water; they can be reused several times. Dry the lid of the and base plate of the instrument with paper towel
26. Remove slides and place in a Coplin jar filled with diluted PBST Wash Buffer at room temperature for 3min
27. Change PBST Wash Buffer and incubate for a further 3 min
28. Counterstain with Hoechst 33342 (1 in 100 in PBS) for 10min
29. Rinse in PBST Wash Buffer for 5min
30. Under subdued lighting, Dehydrate slides through 70%, 85%, and 96% ethanol (2min each)
31. Air dry sections keeping them dark by loosely covering with tin foil
32. Apply an appropriate volume prolong gold anti-fade mountant and mount with a coverslip
33. Ring the coverslips carefully with nail varnish to prevent the sections drying back

5.0 Personal protection –
A Howie coat must be worn at all times.

6.0 Spillages –
Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard. Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with detergent.

7.0 Training –
All staff should undergo training in this technique before performing the procedure.

8.0 Related documents –
8.1 Risk assessments –
RA20138: Immunofluorescence Detection of Antibodies.

9.0 Approval and sign off –

Author:

Name: In Hwa Um

Position: Post Doctorate

Signature:  Date: 05/11/2021

Management Approval:

Name: Peter Mullen

Position: SOP Administrator

Signature:  Date: 05/11/2021

QA release by:

Name: Claire Sneddon

Position: QA Manager

Signature:  Date:11/11/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE