Stellaris® RNA FISH FFPE (Paraffin-Embedded Tissue) Protocol

LGC Biosearch Technologies

Abstract

Stellaris RNA FISH protocol for formalin-fixed, paraffin-embedded (FFPE) tissue.

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Guidelines

Storage Guidelines

Stellaris RNA FISH Probes

Stellaris RNA FISH Probes are shipped dry and can be stored at +2 to +8 °C in this state. Dissolved probe mix should be subjected

to a minimum number of freeze-thaw cycles. For daily and short-term use of dissolved probe mix, storage at +2 to +8 °C in the

dark for up to a month is recommended. For storage lasting longer than a month, we recommend aliquoting and freezing probes

in the dark at -15 to -30 °C.

Stellaris RNA FISH Hybridization Buffer

Stellaris RNA FISH Hybridization Buffer should be stored at +2 to +8 °C for short-term and long-term use.

Stellaris RNA FISH Wash Buffer A and Wash Buffer B

Stellaris RNA FISH Wash Buffers A and B should be stored at room temperature for short-term and long-term use.

Before start

Reagents and Equipment

Reagents and Consumables:

a) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

b) 10X Phosphate Buffered Saline (PBS), RNase-free

c) Ethanol for molecular biology

d) Xylene

e) Nuclease-free water

f) Deionized Formamide

g) Stellaris RNA FISH Hybridization Buffer (Biosearch Technologies Cat# SMF-HB1-10)

h) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)

i) Stellaris RNA FISH Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20)

j) 4’,6-diamidino-2-phenylindole (DAPI)

k) Vectashield® Mounting Medium (Vector Laboratories Cat #H-1000)

l) 24 mm x 60 mm, rectangular, #1 coverglass

m) RNase free consumables such as pipette tips

n) Humidified chamber (or equivalent): 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the

inner chamber edge

o) 37 °C laboratory oven

Microscope:

a) Wide-field fluorescence microscope (e.g., Nikon Eclipse Ti or equivalent). We provide limited support for confocal applications.

b) A high numerical aperture (>1.3) and 60-100x oil-immersion objective.

c) Strong light source, such as a mercury or metal-halide lamp (newer LED-based light sources may also be sufficient).

d) Filter sets appropriate for the fluorophores.

e) Standard cooled CCD camera, ideally optimized for low-light level imaging rather than speed (13 µm pixel size or less is ideal)

Preparation of Reagents

NOTE: When performing Stellaris RNA FISH, it is imperative to limit RNA degradation. Please ensure

that all consumables and

reagents are RNase-free. Recipes below are for set volumes. Please adjust accordingly.

Reconstituting the Dried Probe Stock

ShipReady Probe Set (1 nmol): A ShipReady probe set can provide up to 80 hybridizations. Re-dissolve the dried oligonucleotide

probe blend in 80 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to create a probe stock of 12.5 µM. Mix well by pipetting

up and down, and then vortex and centrifuge briefly.

DesignReady or Custom Probe Set (5 nmol): A DesignReady or custom probe set can provide up to 400 hybridizations.

Re-dissolve the dried oligonucleotide probe blend in 400 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to create a probe

stock of 12.5 µM. Mix well by pipetting up and down, and then vortex and centrifuge briefly.

Hybridization Buffer:

Final composition is 10% (vol./vol.) formamide in Hybridization Buffer

Hybridization Buffer should be mixed fresh for each experiment:

Due to viscosity of the solution, we recommend accounting for a 10% final volume excess in order to have enough Hybridization

Buffer for all of your samples.

For a final volume of 1 mL, mix:

900 µL Stellaris RNA FISH Hybridization Buffer (Biosearch Technologies Cat# SMF-HB1-10)

100 µL Deionized Formamide

NOTE: Do not freeze Hybridization Buffer.

WARNING! Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

WARNING! Be sure to let the formamide warm to room temperature before opening the bottle.

Wash Buffer A (150 mL):

Final composition is 10% (vol./vol.) formamide in Wash Buffer A

Mix and dilute Wash Buffer A fresh for each experiment:

For a final volume of 150 mL, mix:

30 mL Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)

Add 105 mL Nuclease-free water

Add 15 mL Deionized Formamide

Mix well by vortexing gently.

Wash Buffer B:

Add Nuclease-free water to Wash Buffer B bottle upon first use.

Add 88 mL of Nuclease-free water to bottle (Biosearch Technologies Cat# SMF-WB1-20) before use. Mix thoroughly.

Nuclear Stain for use after hybridization:

4’,6-diamidino-2-phenylindole (DAPI) dissolved in Wash Buffer A (see above) at 5 ng/mL to be used in Step I below.

Mounting media:

The commercially available Vectashield Mounting Medium from Vector Laboratories (#H-1000).

Note: For best results, samples mounted with Vectashield Mounting Medium should be imaged the same day.

Materials

- Stellaris® RNA FISH Wash Buffer A SMF-WA1-60 by Biosearch Technologies
- Stellaris® RNA FISH Wash Buffer B SMF-WB1-20 by Biosearch Technologies
- VECTASHIELD Mounting Medium H-1000 by Vector Laboratories
- Stellaris(R) RNA FISH Hybridization Buffer SMF-HB1-10 by Biosearch Technologies

Protocol

Deparaffinization of FFPE tissue sections

Step 1.

NOTES

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NOTE: The method utilized for FFPE tissue preparation, such as harvesting, fixing, and embedding, as well as the age of the FFPE tissue, are major determinants contributing to the success of Stellaris RNA FISH. Namely, care should be taken for the preservation of RNA in the sample by fixing the sample immediately upon dissection. Utilizing small samples, such as core needle biopsies, are ideal due to the rate of formalin penetration. If the level of RNA preservation in the

sample is suspect, we recommend testing RNA integrity prior to performing Stellaris RNA FISH and ensuring that a proper positive control probe set is utilized simultaneously in your experiments.

Deparaffinization of FFPE tissue sections

Step 2.

Paraffin embedded tissue must be sliced at a thickness of 4-10 µm using a microtome and mounted onto a microscope slide.

Deparaffinization of FFPE tissue sections

Step 3.

Immerse the slide-mounted tissue section in 100% xylene for 10 minutes; repeat in fresh 100% xylene for an additional 5 minutes.

DURATION
00:15:00

Deparaffinization of FFPE tissue sections

Step 4.

Immerse the slide in 100% ethanol for 10 minutes; repeat in fresh 100% ethanol for an additional 10 minutes.

DURATION
00:10:00

Deparaffinization of FFPE tissue sections

Step 5.

Immerse the slide in 95% ethanol for 10 minutes.

DURATION
00:10:00

Deparaffinization of FFPE tissue sections

Step 6.

To permeabilize the tissue section, immerse the slide in 70% ethanol for at least 1 hour at room temperature. Slides can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.

DURATION
01:00:00

Deparaffinization of FFPE tissue sections

Step 7.

Immerse the slide in 1X PBS for 2-5 minutes.

DURATION
00:05:00

Deparaffinization of FFPE tissue sections

Step 8.

Decant PBS, and immerse the slide in pre-warmed (37 °C) proteinase K solution (10 µg/mL proteinase K in 1X PBS).

Deparaffinization of FFPE tissue sections

Step 9.

Incubate for 20 minutes at 37 °C.

DURATION
00:20:00

Deparaffinization of FFPE tissue sections

Step 10.

Wash with 1X PBS for 2-5 minutes.

DURATION
00:05:00

Deparaffinization of FFPE tissue sections

Step 11.

Wash with 1X PBS for 2-5 minutes.

DURATION
00:05:00

Hybridization in FFPE embedded tissue sections

Step 12.

If frozen before using, warm the reconstituted probe solution to room temperature. Mix well by vortexing, then centrifuge briefly.

To prepare the Hybridization Buffer containing probe, add 2 µL of probe stock solution to 200 µL of Hybridization Buffer (enough for one coverslip), and then vortex and centrifuge. This creates a working probe solution of 125 nM. This solution will be used on steps 16 and 17.

AMOUNT
200 µl Additional info:

REAGENTS
Stellaris(R) RNA FISH Hybridization Buffer SMF-HB1-10 by Biosearch Technologies

Hybridization in FFPE embedded tissue sections

Step 13.

Immerse the slide-mounted tissue section in Wash Buffer A (see recipe above) for 2-5 minutes.

REAGENTS
Stellaris® RNA FISH Wash Buffer A SMF-WA1-60 by Biosearch Technologies

DURATION
00:05:00

Hybridization in FFPE embedded tissue sections

Step 14.

Assemble humidified chamber: 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the inner chamber edge. This chamber will help prevent evaporation of the probe solution from the tissue section.

Hybridization in FFPE embedded tissue sections

Step 15.

Remove the slide from Wash Buffer A, and carefully wipe away excess buffer surrounding the tissue section.

Hybridization in FFPE embedded tissue sections

Step 16.

Dispense 200 µL of Hybridization Buffer containing probe onto the tissue section of the slide. (Note that 200 µL is recommended when using a 24 mm x 60 mm, rectangular, #1 coverglass. If different sized coverglasses are used, the volume may need to be adjusted accordingly).

AMOUNT

200 µl Additional info:

REAGENTS

Stellaris(R) RNA FISH Hybridization Buffer SMF-HB1-10 by Biosearch Technologies

Hybridization in FFPE embedded tissue sections

Step 17.

Carefully place a clean coverglass over the Hybridization Buffer containing probe to completely cover the tissue section, and allow for even distribution of the Hybridization Buffer. Place the slide in the humidified chamber, cover with the tissue culture lid, and seal with Parafilm®.

Hybridization in FFPE embedded tissue sections

Step 18.

Incubate in the dark at 37 °C for at least 4 hours. (Incubation can be continued up to 16 hours).

DURATION

04:00:00

Hybridization in FFPE embedded tissue sections

Step 19.

Immerse the slide in Wash Buffer A, and allow the submerged coverglass to slide off the tissue section. Gentle agitation may be required to remove the coverglass.

REAGENTS

Stellaris® RNA FISH Wash Buffer A SMF-WA1-60 by Biosearch Technologies

Hybridization in FFPE embedded tissue sections

Step 20.

Incubate in the dark at 37 °C for 30 minutes.

DURATION
00:30:00

Hybridization in FFPE embedded tissue sections

Step 21.

Decant Wash Buffer A, and then add DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.

REAGENTS
Stellaris® RNA FISH Wash Buffer A SMF-WA1-60 by Biosearch Technologies

Hybridization in FFPE embedded tissue sections

Step 22.

Incubate in the dark at 37 °C for 30 minutes.

DURATION
00:30:00

Hybridization in FFPE embedded tissue sections

Step 23.

Decant DAPI staining buffer, and then immerse slide in Wash Buffer B for 2-5 minutes.

REAGENTS
Stellaris® RNA FISH Wash Buffer B SMF-WB1-20 by Biosearch Technologies

DURATION
00:05:00

Hybridization in FFPE embedded tissue sections

Step 24.

Remove the slide from Wash Buffer B, and carefully wipe away excess buffer surrounding the tissue section.

Hybridization in FFPE embedded tissue sections

Step 25.

Add a small drop (approximately 50-100 µL) of Vectashield Mounting Medium onto the tissue section, and cover with a clean #1 coverglass.

AMOUNT
100 µl Additional info:

REAGENTS
VECTASHIELD Mounting Medium H-1000 by Vector Laboratories

Hybridization in FFPE embedded tissue sections

Step 26.

Gently squeeze out excess anti-fade from underneath the coverglass.

Hybridization in FFPE embedded tissue sections

Step 27.

Seal the coverglass perimeter with clear nail polish, and allow to dry.

Hybridization in FFPE embedded tissue sections

Step 28.

Proceed to Imaging

Warnings

WARNING!Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

WARNING! Be sure to let the formamide warm to room temperature before opening the bottle.