Stellaris® RNA FISH Sequential IF + FISH in Adherent Cells Protocol

LGC Biosearch Technologies

Abstract

Stellaris RNA FISH protocol for sequential labeling with IF and RNA FISH in adherent cells.

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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) 37% Formaldehyde Solution

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

d) Nuclease-free water

e) Deionized Formamide

f) Triton X-100

g) Primary antibody

h) Secondary antibody

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

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

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

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

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

n) 18 mm round #1 coverglass

o) 12-well culture plates

p) RNase free consumables such as pipette tips

q) Humidified chamber (or equivalent): 150 mm tissue culture plate; bottom lined evenly with a flat water-saturated paper towel

and a single layer of Parafilm® placed on top of the paper towel

r) Superfrost™ Plus Microscope slides

s) 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.

Fixation Buffer:

Final composition is 3.7% (vol./vol.) formaldehyde in 1X PBS

For a final volume of 10 mL, mix:

1 mL 37% Formaldehyde solution

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

8 mL Nuclease-free water

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 (10 mL):

Final composition is 10% (vol./vol.) formamide in 1X Wash Buffer A

Mix and dilute Wash Buffer A fresh for each experiment:

For a final volume of 10 mL, mix:

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

Add 7 mL Nuclease-free water

Add 1 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) prepared in Wash Buffer A (see above) at 5 ng/mL. This solution is to be used in Step J

below.

Mounting media:

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

Fixation for Sequential IF + FISH in Adherent Cells

Step 1.

Grow cells on 18 mm round #1 coverglass in a 12-well cell culture plate.

NOTES

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NOTE: This protocol has been adapted for a 12-well plate system. To adapt this protocol for your preferred system, volumes should be adjusted accordingly.

Fixation for Sequential IF + FISH in Adherent Cells

Step 2.

Add 1 mL of fixation buffer.

AMOUNT

1 ml Additional info:

PROTOCOL

. Stellaris® RNA FISH Fixation Buffer

CONTACT: LGC Biosearch Technologies

Step 2.1.

37% Formaldehyde solution

AMOUNT

1 ml Additional info:

Step 2.2.

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

AMOUNT

1 ml Additional info:

Step 2.3.

Nuclease-free water

AMOUNT

8 ml Additional info:

Fixation for Sequential IF + FISH in Adherent Cells

Step 3.

Incubate at room temperature for 10 minutes.

DURATION
00:10:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 4.

Wash twice with 1 mL of 1X PBS.

Fixation for Sequential IF + FISH in Adherent Cells

Step 5.

To permeabilize, immerse cells in 1 mL of 0.1% Triton X-100 in 1X PBS for 5 minutes at room temperature.

DURATION
00:05:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 6.

Wash with 1 mL of 1X PBS.

Fixation for Sequential IF + FISH in Adherent Cells

Step 7.

Add 1 mL of appropriately diluted primary antibody in 1X PBS.

Fixation for Sequential IF + FISH in Adherent Cells

Step 8.

Incubate at room temperature for 1 hour.

DURATION
01:00:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 9.

Wash with 1 mL of 1X PBS for 10 minutes, and repeat 2 more times.

DURATION
00:30:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 10.

Add 1 mL of appropriately diluted secondary antibody in 1X PBS.

Fixation for Sequential IF + FISH in Adherent Cells

Step 11.

Incubate at room temperature for 1 hour.

DURATION
01:00:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 12.

Wash with 1 mL of 1X PBS for 10 minutes, and repeat 2 more times.

DURATION
00:30:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 13.

Add 1 mL of fixation buffer.

PROTOCOL

. Stellaris® RNA FISH Fixation Buffer

CONTACT: LGC Biosearch Technologies

Step 13.1.

37% Formaldehyde solution

AMOUNT
1 ml Additional info:

Step 13.2.

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

AMOUNT
1 ml Additional info:

Step 13.3.

Nuclease-free water

AMOUNT
8 ml Additional info:

Fixation for Sequential IF + FISH in Adherent Cells

Step 14.

Incubate at room temperature for 10 minutes.

DURATION
00:10:00

Fixation for Sequential IF + FISH in Adherent Cells

Step 15.

Wash twice with 1 mL of 1X PBS.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 16.

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 1 µL of probe stock solution to 100 µL of Hybridization Buffer, and then vortex and centrifuge (enough for one coverglass). This creates a working probe solution of 125 nM. This solution will be used on steps 20 and 21.

AMOUNT
100 µl Additional info:

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 17.

Aspirate the 1X PBS off the coverglass containing adherent cells within the 12-well plate.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 18.

stellarisAdd 1 mL of Wash Buffer A (see recipe above), and incubate at room temperature for 2-5 minutes.

AMOUNT
1 ml Additional info:

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

DURATION
00:05:00

Hybridization for Sequential IF + FISH in Adherent Cells

Step 19.

Assemble humidified chamber: 150 mm tissue culture plate; bottom lined evenly with a flat water-saturated paper towel and a single layer of Parafilm placed on top of the paper towel. This chamber will help prevent evaporation of the probe solution from under the coverglass.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 20.

Within the humidified chamber, dispense 100 µL of the Hybridization Buffer containing probe onto the Parafilm.

AMOUNT
100 µl Additional info:

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 21.

Gently transfer the coverglass, cells side down, onto the 100 µL drop of Hybridization Buffer containing probe.

AMOUNT

100 µl Additional info:

REAGENTS

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 22.

Cover the humidified chamber with the tissue culture lid, and seal with Parafilm.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 23.

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 for Sequential IF + FISH in Adherent Cells

Step 24.

Gently transfer the coverglass, cells side up, to a fresh 12-well plate containing 1 mL of Wash Buffer A.

AMOUNT

1 ml Additional info:

REAGENTS

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 25.

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

DURATION

00:30:00

Hybridization for Sequential IF + FISH in Adherent Cells

Step 26.

Aspirate Wash Buffer A, and then add 1 mL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.

AMOUNT

1 ml Additional info:

REAGENTS

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 27.

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

DURATION
00:30:00

Hybridization for Sequential IF + FISH in Adherent Cells

Step 28.

Aspirate the DAPI staining buffer, and then add 1 mL of Wash Buffer B. Incubate at room temperature for 2-5 minutes.

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

DURATION
00:05:00

Hybridization for Sequential IF + FISH in Adherent Cells

Step 29.

Add a small drop (approximately 15 µL) of Vectashield Mounting Medium onto a microscope slide, and mount coverglass onto the slide, cells side down.

AMOUNT
15 µl Additional info:

REAGENTS
VECTASHIELD Mounting Medium H-1000 by Vector Laboratories

Hybridization for Sequential IF + FISH in Adherent Cells

Step 30.

Gently wick away excess anti-fade from the perimeter of the coverglass.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 31.

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

Hybridization for Sequential IF + FISH in Adherent Cells

Step 32.

If necessary, gently wipe away any dried salt off the coverglass using water.

Hybridization for Sequential IF + FISH in Adherent Cells

Step 33.
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.