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

LGC Biosearch Technologies

Abstract

Stellaris RNA FISH protocol to simultaneously label adherent cells with IF and RNA FISH.

Citation: LGC Biosearch Technologies Stellaris® RNA FISH Simultaneous IF + FISH in Adherent Cells Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.ek8bczw
Published: 24 Feb 2016

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) Ethanol for molecular biology

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 5X 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 Simultaneous IF + FISH in Adherent Cells

Step 1.

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 Simultaneous IF + FISH in Adherent Cells

Step 2.

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

Fixation for Simultaneous IF + FISH in Adherent Cells

Step 3.

Aspirate growth medium, and wash with 1 mL of 1X PBS.

Fixation for Simultaneous IF + FISH in Adherent Cells

Step 4.

Add 1 mL of fixation buffer.

AMOUNT
1 ml Additional info:

PROTOCOL

. Stellaris® RNA FISH Fixation Buffer

CONTACT: LGC Biosearch Technologies

Step 4.1.

37% Formaldehyde solution

AMOUNT
1 ml Additional info:

Step 4.2.

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

AMOUNT
1 ml Additional info:

Step 4.3.
Nuclease-free water

AMOUNT
8 ml Additional info:

Fixation for Simultaneous IF + FISH in Adherent Cells
Step 5.

Incubate at room temperature for 10 minutes.

DURATION
00:10:00

Fixation for Simultaneous IF + FISH in Adherent Cells
Step 6.

Wash twice with 1 mL of 1X PBS.

Fixation for Simultaneous IF + FISH in Adherent Cells
Step 7.

To permeabilize cells, resuspend cells in 1 mL of 70% ethanol for at least 1 hour at +2 to +8 °C. Cells can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 8.

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 12 and 13.

AMOUNT
100 µl Additional info:

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 9.

Aspirate the 70% ethanol off the coverglass containing adherent cells within the 12-well plate

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 10.

Add 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 Simultaneous IF + FISH in Adherent Cells

Step 11.

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 Simultaneous IF + FISH in Adherent Cells

Step 12.

Within the humidified chamber, dispense 100 µL of the Hybridization Buffer containing probe plus appropriately diluted primary antibody, onto the Parafilm.

AMOUNT

100 µl Additional info:

REAGENTS

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

Hybridization for Simultaneous IF + FISH in Adherent Cells

Step 13.

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

AMOUNT

100 µl Additional info:

REAGENTS

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

Hybridization for Simultaneous IF + FISH in Adherent Cells

Step 14.

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

Hybridization for Simultaneous IF + FISH in Adherent Cells

Step 15.

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 Simultaneous IF + FISH in Adherent Cells

Step 16.

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

AMOUNT
1 ml Additional info:

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 17.

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

DURATION
00:30:00

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 18.

Aspirate Wash Buffer A, and then add 1 mL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) plus appropriately diluted secondary antibody.

AMOUNT
1 ml Additional info:

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 19.

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

DURATION
00:30:00

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 20.

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

AMOUNT
1 ml Additional info:

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

DURATION
00:05:00

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 21.

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 Simultaneous IF + FISH in Adherent Cells
Step 22.

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 23.

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 24.

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

Hybridization for Simultaneous IF + FISH in Adherent Cells
Step 25.
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.