# Stellaris® RNA FISH Alternative Protocol for Adherent Cells

**LGC Biosearch Technologies**

## Abstract

This alternative adherent cell protocol utilizes a methanol based fixation which can be effective at reducing background fluorescence

under certain circumstances.

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## Guidelines

### 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 Solution:

### Final composition is 3:1 Methanol-Glacial Acetic Acid

### Make fresh for each experiment.

### For a final volume of 10 mL, mix:

### 7.5 mL Methanol

### 2.5 mL Glacial Acetic Acid

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.

## Before start

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.

Reagents and Equipment

Reagents and Consumables:

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

b) Methanol

c) Glacial Acetic Acid

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

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) 18 mm round #1 coverglass

m) 12-well culture plates

n) RNase free consumables such as pipette tips

o) 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

p) Superfrost™ Plus Microscope slides

q) 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

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

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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.

## 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

Alternative Fixation of Adherent Cell Lines
**Step 1.**

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

Alternative Fixation of Adherent Cell Lines
**Step 2.**

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

AMOUNT
1 ml Additional info:

Alternative Fixation of Adherent Cell Lines
**Step 3.**

To fix and permeabilize cells, add 1 mL of methanol-acetic acid (MeOH-AcOH) fixation solution.

AMOUNT
1 ml Additional info:

Alternative Fixation of Adherent Cell Lines
**Step 4.**

Incubate at room temperature for 10 minutes.

DURATION
00:10:00

Alternative Fixation of Adherent Cell Lines
**Step 5.**

Cells can be stored at +2 to +8 °C in MeOH-AcOH up to 48 hours before hybridization. Do not use a coverglass containing adherent cells if the MeOH-AcOH has completely evaporated.

Hybridization in Adherent Cells
**Step 6.**

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

## Hybridization in Adherent Cells

**Step 7.**

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 d and e.

## Hybridization in Adherent Cells

**Step 8.**

Aspirate the MeOH-AcOH off the coverglass containing adherent cells within the 12-well plate.

## Hybridization in Adherent Cells

**Step 9.**

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 in Adherent Cells

**Step 10.**

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 in Adherent Cells

**Step 11.**

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 in Adherent Cells

**Step 12.**

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

Hybridization in Adherent Cells

**Step 13.**

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

Hybridization in Adherent Cells

**Step 14.**

Incubate in the dark at 37 °C for 2 hours. (Incubation can be continued up to 16 hours).

DURATION

02:00:00

Hybridization in Adherent Cells

**Step 15.**

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 in Adherent Cells

**Step 16.**

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

DURATION

00:30:00

Hybridization in Adherent Cells

**Step 17.**

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 in Adherent Cells

**Step 18.**

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

DURATION

00:30:00

Hybridization in Adherent Cells

**Step 19.**

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 in Adherent Cells
**Step 20.**

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 in Adherent Cells
**Step 21.**

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

Hybridization in Adherent Cells
**Step 22.**

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

Hybridization in Adherent Cells
**Step 23.**

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

Hybridization in Adherent Cells
**Step 24.**
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.