# Stellaris® RNA FISH Protocol for Adherent Cells

**LGC Biosearch Technologies**

## Abstract

A set of Stellaris FISH Probes comprises up to 48 singly labeled oligonucleotides designed to selectively bind to targeted transcripts. Stellaris FISH Probes bound to target RNA produce fluorescent signals that permit detection of single RNA molecules as diffraction-limited spots by conventional fluorescence microscopy. See the Biosearch website for additional details.

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## Guidelines

### General Protocol & Storage

### Product Description

A set of Stellaris RNA FISH Probes is comprised of up to 48 singly labeled oligonucleotides designed to selectively bind to targeted transcripts. Stellaris RNA FISH Probes bound to target RNA produce fluorescent signals that permit detection of single RNA molecules as diffraction-limited spots by conventional fluorescence microscopy.

### 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) 18 mm round #1 coverglass
b) 12-well culture plates
c) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
d) 37% Formaldehyde Solution
e) 10X Phosphate Buffered Saline (PBS), RNase-free
f) Nuclease-free water
g) Deionized Formamide
h) Ethanol for molecular biology
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) RNase-free consumables such as pipette tips
m) 4’,6-diamidino-2-phenylindole (DAPI)
n) Vectashield® Mounting Medium (Vector Laboratories Cat #H-1000)
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) 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 (Xenon or LED are typically not bright enough).
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 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 1mL 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.

- *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.

**REFERENCES**

1. Raj, A., van den Bogaard, P., Rifkin S.A., van Oudenaarden, A., and Tyagi, S. Imaging individual mRNA molecules using multiple singly labeled probes. Nat. Methods. 2008; 5: 877-9

2. Femino, A.M., Fay, F.S., Fogarty, K., and Singer, R.H. Visualization of single RNA transcripts in situ. Science 1998; 280: 585–90.

## 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
- Stellaris® RNA FISH Hybridization Buffer SMF-HB1-10 by Biosearch Technologies

VECTASHIELD Mounting Medium H-1000 by Vector Laboratories

## Protocol

Fixation of Adherent Cells

**Step 1.**

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

Fixation of Adherent Cells

**Step 2.**

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

AMOUNT

1 ml Additional info:

Fixation of Adherent Cells

**Step 3.**

Add 1 mL of fixation buffer.

AMOUNT

1 ml Additional info:

PROTOCOL

**. Stellaris® RNA FISH Fixation Buffer**

CONTACT: LGC Biosearch Technologies

**Step 3.1.**

37% Formaldehyde solution

AMOUNT

1 ml Additional info:

**Step 3.2.**

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

AMOUNT

1 ml Additional info:

**Step 3.3.**

Nuclease-free water

AMOUNT

8 ml Additional info:

## Fixation of Adherent Cells

**Step 4.**

Incubate at room temperature for 10 minutes.

DURATION

00:10:00

## Fixation of Adherent Cells

**Step 5.**

Wash with 1 mL of 1X PBS.

AMOUNT

1 ml Additional info:

## Fixation of Adherent Cells

**Step 6.**

Wash again with 1 mL of 1X PBS.

AMOUNT

1 ml Additional info:

## Fixation of Adherent Cells

**Step 7.**

To permeabilize, immerse cells in 1 mL of 70% (vol./vol.) ethanol for at least 1 hour at +2 to +8 °C.

AMOUNT

1 ml Additional info:

DURATION

01:00:00

NOTES

**LGC Biosearch Technologies** 12 Mar 2015

Cells can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridization.

## Hybridization in Adherent Cells

**Step 8.**

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

## Hybridization in Adherent Cells

**Step 9.**

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, which is enough for one coverslip. This creates a working probe solution of 125 nM. This solution will be used in the steps below.

NOTES

**LGC Biosearch Technologies** 17 Jul 2014
Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

**LGC Biosearch Technologies** 17 Jul 2014
Be sure to let the formamide and Hybridization Buffer warm to room temperature before opening the bottle.

**LGC Biosearch Technologies** 17 Mar 2015
Do not freeze Hybridization Buffer.

## Hybridization in Adherent Cells

**Step 10.**
Aspirate the 70% ethanol off the coverglass containing adherent cells within the 12-well plate.

## Hybridization in Adherent Cells

**Step 11.**
Add 1 mL of Wash Buffer A (see recipe in guidelines), 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 12.**
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.

NOTES
**LGC Biosearch Technologies** 17 Jul 2014
This chamber will help prevent evaporation of the probe solution from under the coverglass.

## Hybridization in Adherent Cells

**Step 13.**
Within the humidified chamber, dispense 100 µL of the Hybridization Buffer containing probe onto the Parafilm.

## Hybridization in Adherent Cells

**Step 14.**
Gently transfer the coverglass, cells side down, onto the 100 µL drop of hybridization buffer containing probe.

## Hybridization in Adherent Cells

**Step 15.**
Cover the humidified chamber with the tissue culture lid, and seal with Parafilm.

## Hybridization in Adherent Cells

**Step 16.**
Incubate in the dark at 37 °C for at least 4 hours.

DURATION
04:00:00

NOTES
**LGC Biosearch Technologies** 17 Jul 2014

Incubation can be continued up to 16 hours.

Hybridization in Adherent Cells

**Step 17.**

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 18.**

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

DURATION

00:30:00

Hybridization in Adherent Cells

**Step 19.**

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

Hybridization in Adherent Cells

**Step 20.**

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

DURATION

00:30:00

Hybridization in Adherent Cells

**Step 21.**

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 22.**

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 23.**

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

Hybridization in Adherent Cells

**Step 24.**
Seal the coverglass perimeter with clear nail polish, and allow to dry.

Hybridization in Adherent Cells

**Step 25.**
If necessary, gently wipe away any dried salt off the coverglass with water.

**Step 26.**
Proceed to imaging.

## Warnings

When performing Stellaris RNA FISH, it is imperative to limit RNA degradation. Please ensure that all consumables and reagents are RNase-free.