Stellaris® RNA FISH 96 Well Glass Bottom Plate Protocol

LGC Biosearch Technologies

Abstract

This protocol is specifically designed for high throughput applications of Stellaris in 96 well glass bottom plates.

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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) Methanol

c) Glacial Acetic Acid

d) 37% Formaldehyde Solution

e) Ethanol for molecular biology

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

g) Nuclease-free water

h) Deionized Formamide

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

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

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

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

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

n) 96-well glass bottom cell culture plates with #0 or #1 coverglass*

o) Mineral Oil

p) RNase free consumables such as pipette tips

q) 37 °C laboratory oven

*Cell culture plate must be resistant to the fixation, wash buffers, and microscope objective immersion oil used in this protocol

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 50 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 250 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.

Standard Fixation Solution:

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

WARNING! Formaldehyde is a known carcinogen and should be used in a chemical fume hood. Please refer to the appropriate

SDS (Safety Data Sheet) prior to use.

Alternative Fixation Solution:

Final composition is 3:1 Methanol-Glacial Acetic Acid

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 (LGC 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.

Please consult the appropriate SDS (Safety Data Sheet) prior to use

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

WARNING! Formaldehyde is a known human carcinogen and should be use in a chemical fume hood. Please consult the

appropriate SDS (Safety Data Sheet) prior to use

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 (LGC 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 (LGC 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. 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

Standard Fixation

Step 1.

Grow cells in a 96-well glass bottom cell culture plate

NOTES

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NOTE: This protocol has been adapted for a 96 well glass bottom system. To adapt this protocol for your preferred system, volumes should be adjusted accordingly.

Standard Fixation

Step 2.

Decant growth medium, and wash with 200 µL of 1X PBS

AMOUNT

200 µl Additional info:

Standard Fixation

Step 3.

To fix cells, add 200 µL of 3.7% Formaldehyde fixation solution

AMOUNT

200 µl Additional info:

Standard Fixation

Step 4.

Incubate at room temperature for 10 minutes

DURATION
00:10:00

Standard Fixation

Step 5.

Wash with 200 µL of 1X PBS

AMOUNT
200 µl Additional info:

Standard Fixation

Step 6.

Wash with 200 µL of 1X PBS

AMOUNT
200 µl Additional info:

Alternative Fixation

Step 7.

The Alternative Fixation steps are an alternative to the standard fixation and are not meant to be sequential.

Alternative Fixation

Step 8.

Grow cells in a 96-well glass bottom cell culture plate

Alternative Fixation

Step 9.

Decant growth media, and wash with 200 µL of 1X PBS

AMOUNT
200 µl Additional info:

Alternative Fixation

Step 10.

To fix and permeabilize cells, add 200 µL of methanol-acetic acid (MeOH-AcOH) fixation solution

AMOUNT
200 µl Additional info:

Alternative Fixation

Step 11.

Incubate at room temperature for 10 minutes

DURATION
00:10:00

Alternative Fixation

Step 12.

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

Hybridization in Adherent Cells

Step 13.

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

To prepare the Hybridization Buffer containing probe, add 1.5 µL of probe stock solution to 75 µL of Hybridization Buffer, and then vortex

and centrifuge (enough for one well). This creates a working probe solution of 250 nM. This solution will be used on step 17.

AMOUNT
75 µl Additional info:

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

Hybridization in Adherent Cells

Step 14.

Decant MeOH-AcOH or 70% ethanol from wells containing adherent cells

Hybridization in Adherent Cells

Step 15.

Add 200 µL of Wash Buffer A (see recipe above), and incubate at room temperature for 2-5 minutes

AMOUNT
200 µl Additional info:

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

DURATION
00:05:00

Hybridization in Adherent Cells

Step 16.

Decant Wash Buffer A

Hybridization in Adherent Cells

Step 17.

Add 75 µL of Hybridization Buffer containing Probe into each well

AMOUNT
75 µl Additional info:

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

Hybridization in Adherent Cells
Step 18.

Incubate in the dark at 37 °C for 4 to 16 hours

a) Incubation is recommended for 16 hours using the Standard Fixation Method

b) Incubation is recommended for 2 hours using the Alternative Fixation Method

DURATION
16:00:00

Hybridization in Adherent Cells
Step 19.

Aspirate the Hybridization Buffer containing Probe, and add 200 µL of Wash Buffer A

AMOUNT
200 µl Additional info:

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

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.

Decant Wash Buffer A, and then add 200 µL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei

AMOUNT
200 µl Additional info:

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

Hybridization in Adherent Cells
Step 22.

Incubate in the dark at 37 °C for 30 minutes

DURATION
00:30:00

Hybridization in Adherent Cells

Step 23.

Decant DAPI staining buffer, and then add 200 µL 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 in Adherent Cells

Step 24.

Add 30 µL of VectaShield Mounting Medium to the well and top with 30 µL of Mineral Oil

AMOUNT
30 µl Additional info:

REAGENTS
VECTASHIELD Mounting Medium H-1000 by Vector Laboratories

Hybridization in Adherent Cells

Step 25.
Proceed to Imaging

Warnings

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