Stellaris® RNA FISH Cells in Suspension Protocol

LGC Biosearch Technologies

Abstract

Stellaris® RNA FISH Cells in Suspension Protocol is specifically optimized to allow to visualization of single RNA transcripts in suspended, fixed 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) Ethanol for molecular biology

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 x 18 mm square #1 coverglass

m) RNase free consumables such as pipette tips

n) Superfrost™ Plus Microscope slides

o) Kimwipes™

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 (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 I

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 of Cells in Suspension

Step 1.

Centrifuge suspension cells (2 – 5 x 106 cells) in a 15 mL conical tube.

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NOTE: All centrifugations are performed at 200 x g at room temperature in a table top centrifuge for 2 minutes. This protocol is

adapted from Chapter 1 of Nuclear Bodies and Noncoding RNAs, Methods in Molecular Biology3.

Fixation of Cells in Suspension

Step 2.

Aspirate supernatant, leaving cells in a pellet at base of tube.

Fixation of Cells in Suspension

Step 3.

Gently resuspend cells in 1 mL of 1X PBS, and centrifuge to pellet cell suspension.

AMOUNT
1 ml Additional info:

Fixation of Cells in Suspension

Step 4.

Aspirate the 1X PBS, and gently resuspend cells in 1 mL of fixation buffer. Mix well by pipetting or inverting the tube.

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 of Cells in Suspension

Step 5.

Incubate at room temperature for 10 minutes.

DURATION

00:10:00

Fixation of Cells in Suspension

Step 6.

Centrifuge to pellet cell suspension. Aspirate fixation buffer, and wash cells three times with 1 mL of 1X PBS. Mix well by gently pipetting up and down to resuspend pellet.

AMOUNT

3 ml Additional info:

Fixation of Cells in Suspension

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.

AMOUNT

1 ml Additional info:

DURATION

01:00:00

Hybridization of Cells in Suspension

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 step 13.

AMOUNT

100 µl Additional info:

REAGENTS

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

Hybridization of Cells in Suspension

Step 9.

Invert tube with fixed and permeabilized suspension cells several times to resuspend cells. Then place 50- 500 µL of cells (depending on concentration) in a microcentrifuge tube. Alternatively, at this step you can use poly-L-lysine or cytospin to adhere the fixed and permeabilized suspension cells to a round #1 coverglass after which you can perform RNA FISH following the Adherent Cell Protocol.

Hybridization of Cells in Suspension

Step 10.

Centrifuge to pellet cells and aspirate 70% ethanol.

Hybridization of Cells in Suspension

Step 11.

Gently resuspend cells in 500 µL of Wash Buffer A (see recipe above).

AMOUNT
500 µl Additional info:

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

Hybridization of Cells in Suspension

Step 12.

Centrifuge to pellet cells and aspirate Wash Buffer A.

Hybridization of Cells in Suspension

Step 13.

Resuspend cells in 100 µL of Hybridization Buffer containing probe. Mix well by pipetting up and down.

AMOUNT
100 µl Additional info:

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

Hybridization of Cells in Suspension

Step 14.

Incubate microcentrifuge tube in the dark at 37 °C overnight (16 hours).

DURATION
16:00:00

Hybridization of Cells in Suspension

Step 15.

Centrifuge to pellet cells and aspirate about 50% of the Hybridization Buffer containing probe. The pellet is very fluffy and easy to lose at this point.

Hybridization of Cells in Suspension

Step 16.

Add 500 µL of Wash Buffer A. Centrifuge to pellet cells and aspirate solution. Be careful not to disturb the pellet.

AMOUNT
500 µl Additional info:

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

Hybridization of Cells in Suspension
Step 17.

Resuspend cells in 500 µL of Wash Buffer A.

AMOUNT
500 µl Additional info:

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

Hybridization of Cells in Suspension
Step 18.

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

DURATION
00:30:00

Hybridization of Cells in Suspension
Step 19.

Centrifuge to pellet cells and aspirate Wash Buffer A.

Hybridization of Cells in Suspension
Step 20.

Resuspend cells in 500 µL of DAPI nuclear stain (1X Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.

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

Hybridization of Cells in Suspension
Step 21.

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

DURATION
00:30:00

Hybridization of Cells in Suspension
Step 22.

Centrifuge to pellet cells and aspirate DAPI nuclear stain.

Hybridization of Cells in Suspension
Step 23.

Resuspend cells in 500 µL of Wash Buffer B.

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

Hybridization of Cells in Suspension
Step 24.

Centrifuge to pellet cells and aspirate Wash Buffer B. Resuspend cells in a small drop (approximately 30 µL) of Vectashield Mounting Medium.

AMOUNT
30 µl Additional info:

REAGENTS
VECTASHIELD Mounting Medium H-1000 by Vector Laboratories

Hybridization of Cells in Suspension
Step 25.

Place 5-10 µL of cell suspension on a clean glass microscope slide and then place an 18 x 18 mm square #1 coverglass over the cells to spread the solution.

Hybridization of Cells in Suspension
Step 26.

Place a Kimwipe over the coverglass and apply gentle pressure over the surface of the coverglass, pressing it firmly onto the surface of the slide. While applying pressure, be careful not to move the coverglass horizontally as this could result in sheared cells. The Kimwipe will wick up excess mounting medium.

Hybridization of Cells in Suspension
Step 27.

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

Hybridization of Cells in Suspension
Step 28.
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.