Stellaris® RNA FISH Protocol for Brain

LGC Biosearch Technologies

Abstract

Stellaris® RNA FISH protocol for fresh frozen mouse brain

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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) 20% Electron Microscopy Grade Formaldehyde

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

d) Nuclease-free water

e) Ethanol for molecular biology

f) Triethanolamine

g) Acetic Anhydride

h) Chloroform

i) Deionized Formamide

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

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

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

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

n) Prolong® Gold Antifade Mountant (ThermoFisher Scientific Cat #P36930)

o) 24 x 60 mm rectangular coverglass

p) RNase free consumables such as pipette tips

q) Humidified chamber (or equivalent): 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the

inner chamber edge

r) 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 50 mL, mix:

1 mL 20% Formaldehyde solution

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

35 mL Nuclease-free water

TEA Buffer (10X):

NOTE: This buffer is light sensitive. It is suggested to wrap reagent bottle in foil.

For a final volume of 100 mL, mix:

13.3 mL Triethanolamine

60 mL Nuclease-free water H2O

Then bring to pH 8.0 using HCL (~5-10 mL of HCL)

Once at pH 8.0, add water to 100 mL final volume

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.

Wash Buffer A (50 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 50 mL, mix:

10 mL Stellaris RNA FISH 5X Wash Buffer A (LGC Biosearch Technologies Cat# SMF-WA1-60)

Add 5 mL Deionized Formamide

Add 35 mL Nuclease-free water

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) prepared in Wash Buffer A (see above) at 5 ng/mL. This solution is to be used in Step J

below.

Mounting media:

Prolong Gold Antifade Mountant (ThermoFisher Scientific Cat #P36930).

NOTE: Samples mounted with Prolong Gold should be allowed to cure overnight at room temperature and then imaged

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

Prolong Gold P36930 by Thermo Fisher Scientific

Protocol

Slide Prep and Hybridization

Step 1.

Thaw the slide-mounted tissue section to room temperature.

Slide Prep and Hybridization

Step 2.

Immerse the slide in cold 4% E.M. grade paraformaldehyde in 1X PBS for 15 minutes.

DURATION
00:15:00

Slide Prep and Hybridization

Step 3.

Wash with 1X PBS for 5 minutes.

DURATION
00:05:00

Slide Prep and Hybridization

Step 4.

Wash twice with 1X PBS for 5 minutes.

DURATION
00:05:00

Slide Prep and Hybridization

Step 5.

Dip the slide in nuclease-free water.

Slide Prep and Hybridization

Step 6.

Dip the slide in 1X TEA buffer.

Slide Prep and Hybridization

Step 7.

Immerse the slide in 1X TEA + Acetic Anhydride for 10 minutes (Stirring!) ***

DURATION
00:10:00

NOTES
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Note: Acetic Anhydride must be added fresh each time. Add Acetic Anhydride to fresh 1X TEA buffer after the second 1X PBS

wash in step 3, about 1 minute before dipping slides in DEPC water. For 50 mL of 1X TEA, add 63 µL of Acetic Anhydride.

Slide Prep and Hybridization

Step 8.

Immerse the slide in 2X SSC for 3 minutes.

DURATION
00:03:00

Slide Prep and Hybridization

Step 9.

Immerse the slide in 70% ethanol for 3 minutes.

DURATION
00:03:00

Slide Prep and Hybridization

Step 10.

Immerse the slide in 95% ethanol for 3 minutes.

DURATION
00:03:00

Slide Prep and Hybridization

Step 11.

Immerse the slide in 100% ethanol for 3 minutes.

DURATION
00:03:00

Slide Prep and Hybridization

Step 12.

Immerse the slide in Chloroform for 5 minutes.

DURATION
00:05:00

Slide Prep and Hybridization

Step 13.

3Immerse the slide in 100% ethanol for 3 minutes.

DURATION
00:05:00

Slide Prep and Hybridization

Step 14.

Immerse the slide in 95% ethanol for 3 minutes.

DURATION
00:03:00

Slide Prep and Hybridization

Step 15.

Let air dry for 90+ minutes (but no longer than 4 hours).

DURATION
01:30:00

Hybridization in frozen tissue sections

Step 16.

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

To prepare the hybridization solution, add 4.0 µL of probe stock solution to 200 µL of hybridization buffer, and then vortex and centrifuge.

This creates a working probe solution of 250 nM. This solution will be used on step 18.

Hybridization in frozen tissue sections

Step 17.

Assemble a humidified chamber: 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the inner chamber edge. This chamber will help prevent evaporation of the probe solution from the tissue section.

Hybridization in frozen tissue sections

Step 18.

After slide has dried for 90+ minutes, dispense 200 µL of hybridization buffer containing probe onto the tissue sections of the slide.

AMOUNT
200 µl Additional info:

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

Hybridization in frozen tissue sections
Step 19.

Carefully place a clean 24 x 60 mm rectangular coverglass over the hybridization solution to completely cover the tissue sections and allow for even distribution of the hybridization solution. Place the slide in the humidified chamber, cover with the tissue culture lid, and seal chamber with parafilm.

Hybridization in frozen tissue sections
Step 20.

Incubate in the dark at 37 °C for at least 4 hours (incubation can be continued up to 16 hours).

DURATION
04:00:00

In Situ Washes
Step 21.

Immerse the slide in wash buffer A, and allow the submerged coverglass to slide off the tissue section. Gentle agitation may

be required to remove the coverglass.

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

In Situ Washes
Step 22.

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

DURATION
00:30:00

In Situ Washes
Step 23.

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

In Situ Washes
Step 24.

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

DURATION

00:30:00

In Situ Washes

Step 25.

Decant DAPI staining buffer, and then immerse slide in Wash Buffer B for 3 minutes.

REAGENTS

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

DURATION

00:03:00

In Situ Washes

Step 26.

Immerse slide in 50% ethanol for 3 minutes.

DURATION

00:03:00

In Situ Washes

Step 27.

Immerse slide in 85% ethanol for 3 minutes.

DURATION

00:03:00

In Situ Washes

Step 28.

Immerse slide in 100% ethanol for 3 minutes.

DURATION

00:03:00

In Situ Washes

Step 29.

Let air dry for 5-10 minutes.

DURATION

00:10:00

In Situ Washes

Step 30.

Add a drop or two (approximately 50-100 µL) of Prolong Gold Antifade Mountant onto the tissue sections. Cover with a clean 24 x 60 mm coverglass, allowing the antifade to spread evenly across the tissue sections

AMOUNT

100 µl Additional info:

REAGENTS

Prolong Gold P36930 by Thermo Fisher Scientific

In Situ Washes

Step 31.

Allow Prolong Gold to cure overnight, in the dark, at room temperature.

In Situ Washes

Step 32.

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

In Situ Washes

Step 33.

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.

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.