Stellaris RNA FISH

Protocol for frozen tissue

General protocol and 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 Hybridisation Buffer

Stellaris RNA FISH Hybridisation 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.

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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) Ethanol for molecular biology
e) Nuclease-free water
f) Deionised Formamide
g) Stellaris RNA FISH Hybridisation Buffer (LGC, Biosearch Technologies Cat# SMF-HB1-10, SMF-HB1-100)
h) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60, SMF-WA1-600)
i) Stellaris RNA FISH Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20, SMF-WB1-200)
j) 4',6-diamidino-2-phenylindole (DAPI)
k) Vectashield® Mounting Medium (Vector Laboratories Cat #H-1000)
l) CoverGrip™ Coverslip Sealant (Biotium Cat# 23005) or clear nail polish
m) 24 mm × 60 mm, rectangular, #1 coverglass
n) RNase free consumables such as pipette tips
o) Humidified chamber (or equivalent): 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the inner chamber edge
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 or sCMOS camera, ideally optimised for low-light level imaging rather than speed (13 µm pixel size or less is ideal).

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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 two set volumes (1X and 10X). Please adjust accordingly.

Reconstituting the dried probe stock:

ShipReady Probe Set (1 nmol):

- A ShipReady probe set can provide up to 80 hybridisations. 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 hybridisations. 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 (500 mL), mix:

- 5 mL (50 mL) 37% Formaldehyde solution
- 5 mL (50 mL) 10X Phosphate Buffered Saline (PBS), RNase-free
- 40 mL (400 mL) Nuclease-free water

WARNING! Formaldehyde 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.

Hybridisation Buffer:

Final composition is 10% (vol./vol.) formamide in Hybridisation Buffer

Hybridisation 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 Hybridisation Buffer for all of your samples.

For a final volume of 1 mL (10 mL), mix:

- 900 µL (9 mL) Stellaris RNA FISH Hybridisation Buffer.
- 100 µL (1 mL) Deionised Formamide. Mix thoroughly by vortexing and pipetting up and down.

NOTE: Do not freeze Hybridisation 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.

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Wash Buffer A (150 mL/1500 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 150 mL (1500 mL), mix:

- 30 mL (300 mL) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)
- Add 15 mL (150 mL) Deionised Formamide
- Add 105 mL (1050 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 (880 mL) of Nuclease-free water to bottle before use.
- Mix thoroughly.

Nuclear Stain for use after hybridisation:

- 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 i below.

Mounting media:

- Vectashield Mounting Medium from Vector Laboratories

NOTE: For best results, samples mounted with Vectashield Mounting Medium should be imaged the same day.

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NOTE: The method utilised for frozen tissue preparation, such as freezing immediately upon dissection, storage at -80 °C, as well as frozen sectioning utilising a cryostat, are major determinants contributing to the success of Stellaris RNA FISH. Namely, care should be taken for the preservation of RNA in the sample. If the level of RNA preservation in the sample is suspect, please perform RNA integrity testing prior to performing Stellaris RNA FISH and ensure that a proper positive control probe set is utilised simultaneously in your experiments.

Fixation of frozen tissue sections

a) Slice frozen tissue at a thickness of 4-10 µm using a cryostat and mount onto a microscope slide.
b) Thaw the slide-mounted tissue section to room temperature.
c) Immerse the slide in fixation buffer for 10 minutes at room temperature.
d) Wash twice with 1X PBS for 2-5 minutes.
e) To permeabilise the tissue section, immerse the slide in 70% (vol./vol.) ethanol for at least 1 hour at room temperature. Slides can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridisation.

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Hybridisation in frozen tissue sections

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

To prepare the Hybridisation Buffer containing probe, add 2 µL of probe stock solution to 200 µL of Hybridisation Buffer (enough for one coverslip), and then vortex and centrifuge. This creates a working probe solution of 125 nM. This solution will be used on steps d and e.

a) Immerse the slide-mounted tissue section in Wash Buffer A (see recipe above) for 2-5 minutes.
b) Assemble 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.
c) Remove the slide from Wash Buffer A, and carefully wipe away excess buffer surrounding the tissue section.
d) Dispense 200 µL of Hybridisation Buffer containing probe onto the tissue section of the slide. (Note that 200 µL is recommended when using a 24 mm × 60 mm, rectangular, #1 coverglass. If different sized coverglasses are used, the volume may need to be adjusted accordingly).
e) Carefully place a clean coverglass over the Hybridisation Buffer containing probe to completely cover the tissue section, and allow for even distribution of the Hybridisation Buffer. Place the slide in the humidified chamber, cover with the tissue culture lid, and seal with Parafilm®.
f) Incubate in the dark at 37 °C for at least 4 hours. (Incubation can be continued up to 16 hours).
g) 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.
h) Incubate in the dark at 37 °C for 30 minutes.
i) Decant Wash Buffer A, and then add DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.
j) Incubate in the dark at 37 °C for 30 minutes.
k) Decant DAPI staining buffer, and then immerse slide in Wash Buffer B for 2/5 minutes.
l) Remove the slide from Wash Buffer B, and carefully wipe away excess buffer surrounding the tissue section.
m) Add a small drop (approximately 50-100 µL) of Vectashield Mounting Medium onto the tissue section, and cover with a clean #1 coverglass.
n) Gently squeeze out excess anti-fade from underneath the coverglass.
o) Seal the coverglass perimeter with CoverGrip (or clear nail polish), and allow to dry.

Proceed to imaging.

References

1. Tesch, G.H., Lan, H.Y., and Nikolic-Paterson, D.J. Treatment of tissue sections for *in situ* hybridisation. Methods Mol. Biol. 2006; 326, 1-7. doi: 10.1385/1-59745-007-3:1
2. 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, 877-879. doi: 10.1038/nmeth.1253
3. Femino, A.M., Fay, F.S., Fogarty, K., and Singer, R.H. Visualisation of single RNA transcripts *in situ*. Science 1998; 280, 585-590. doi: 10.1126/science.280.5363.585

Guidelines citing the use of Stellaris RNA FISH Probes and methods in scientific publications

Please acknowledge the use of Stellaris RNA FISH Probes and/or protocols in the experimental **Materials and Methods** or **Methods** section of your manuscript. Refer to the following examples as guidelines for proper citation of the Stellaris RNA FISH Probe sets and/or protocols:

Citing catalogued probe sets:

"Stellaris™ RNA FISH Probes recognising <catalogued gene set name> and labelled with Quasar™ 570 dye (Catalog #, LGC, Biosearch Technologies, Petaluma, CA) were hybridised to <samples>, following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing Custom Probe sets designed with the Stellaris FISH Probe Designer:

"Custom Stellaris™ RNA FISH Probes were designed against <your RNA of interest (include accession number and nucleotides covered if relevant)> by utilising the Stellaris RNA FISH Probe Designer (LGC, Biosearch Technologies, Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). The <samples> were hybridised with the <your RNA of interest> Stellaris RNA FISH Probe set labelled with <your dye of choice> (Biosearch Technologies), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing Custom Probe sets utilising previously published sequences:

"Custom Stellaris™ RNA FISH Probes recognising <your RNA of interest (include accession number and nucleotides covered if relevant)> and labelled with <your dye of choice>, were purchased from LGC, Biosearch Technologies (Petaluma, CA). Probe set sequences utilised in the experiments have been previously described <cite published manuscript>. The <samples> were hybridised with the <your RNA of interest> Stellaris RNA FISH Probe set, following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

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Citing 3′ Amine Oligos in plates used for Stellaris RNA FISH designed with the Stellaris FISH Probe Designer:

"Custom 3′ amine oligos in plates were designed against <your RNA of interest (include accession number and nucleotides covered if relevant)> by utilising the Stellaris™ RNA FISH Probe Designer (LGC, Biosearch Technologies, Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). Probes were labelled with <your dye of choice> using <insert your labellling protocol or citation of previously published labelling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Citing 3′ Amine Oligos in plates used for Stellaris RNA FISH using previously published sequences:

"Custom 3′ amine oligos in plates recognising <your RNA of interest (include accession number and nucleotides covered if relevant)> were purchased from LGC, Biosearch Technologies (Petaluma, CA). Probe set sequences utilised in the experiments have been previously described <cite published manuscript>. Probes were labelled with <your dye of choice> using <insert your labelling protocol or citation of previously published labelling protocol>. The <samples> were hybridised with the <your RNA of interest> oligonucleotides (Biosearch Technologies), following the manufacturer's instructions available online at www.biosearchtech.com/stellarisprotocols. Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>."

Technical support

If you require additional information or technical assistance, please feel free to email our Technical Support Team at: techsupport@lgcgroup.com.

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