

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) TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- b) 10X Phosphate Buffered Saline (PBS), RNase-free
- c) Ethanol for molecular biology
- d) Xylene
- e) Nuclease-free water
- f) Deionized Formamide
- 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) 24 mm x 60 mm, rectangular, #1 coverglass
- m) RNase free consumables such as pipette tips
- n) Humidified chamber (or equivalent): 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the inner chamber edge
- o) 37 °C laboratory oven

Microscope:

- Wide-field fluorescence microscope (e.g., Nikon Eclipse Ti or equivalent). We provide limited support for confocal applications.
- A high numerical aperture (>1.3) and 60-100x oil-immersion objective.
- Strong light source, such as a mercury or metal-halide lamp (newer LED-based light sources may also be sufficient).
- Filter sets appropriate for the fluorophores.
- 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.

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 (150 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**, mix:

- 30 mL Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)
- Add 105 mL Nuclease-free water
- Add 15 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) dissolved in Wash Buffer A (see above) at 5 ng/mL to be used in Step I below.

Mounting media:

The commercially available Vectashield Mounting Medium from Vector Laboratories (#H-1000).

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

Protocol for Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

NOTE: The method utilized for FFPE tissue preparation, such as harvesting, fixing, and embedding, as well as the age of the FFPE tissue, are major determinants contributing to the success of Stellaris RNA FISH. Namely, care should be taken for the preservation of RNA in the sample by fixing the sample immediately upon dissection. Utilizing small samples, such as core needle biopsies, are ideal due to the rate of formalin penetration. If the level of RNA preservation in the sample is suspect, we recommend testing RNA integrity prior to performing Stellaris RNA FISH and ensuring that a proper positive control probe set is utilized simultaneously in your experiments.

Deparaffinization of FFPE tissue sections

- a) Paraffin embedded tissue must be sliced at a thickness of 4-10 μm using a microtome and mounted onto a microscope slide.
- b) Immerse the slide-mounted tissue section in 100% xylene for 10 minutes; repeat in fresh 100% xylene for an additional 5 minutes.
- c) Immerse the slide in 100% ethanol for 10 minutes; repeat in fresh 100% ethanol for an additional 10 minutes.
- d) Immerse the slide in 95% ethanol for 10 minutes.
- e) To permeabilize the tissue section, immerse the slide in 70% ethanol for at least 1 hour at room temperature. Slides can be stored at +2 to +8 $^{\circ}\text{C}$ in 70% ethanol up to a week before hybridization.
- f) Immerse the slide in 1X PBS for 2-5 minutes.
- g) Decant PBS, and immerse the slide in pre-warmed (37 $^{\circ}\text{C}$) proteinase K solution (10 $\mu\text{g}/\text{mL}$ proteinase K in 1X PBS).
- h) Incubate for 20 minutes at 37 $^{\circ}\text{C}$.
- i) Wash twice with 1X PBS for 2-5 minutes.

Hybridization in FFPE embedded tissue sections

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 2 μL of probe stock solution to 200 μL of Hybridization 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 Hybridization Buffer containing probe onto the tissue section of the slide. (Note that 200 μL is recommended when using a 24 mm x 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 Hybridization Buffer containing probe to completely cover the tissue section, and allow for even distribution of the Hybridization 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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ 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 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 hybridization. *Methods Mol. Biol.* 2006; 326 1-7..
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, 877-9.
3. Femino, A.M., Fay, F.S., Fogarty, K., and Singer, R.H. Visualization of single RNA transcripts in situ. *Science* 1998; 280: 585–590.

Guidelines for 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 ShipReady and DesignReady Probe Sets: “Stellaris® FISH Probes recognizing <catalogued gene set name> and labeled with <your dye of choice> (Catalog #, Biosearch Technologies, Inc., Petaluma, CA) were hybridized 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® RNAFISH Probe Designer: “Custom Stellaris® RNA FISH Probes were designed against <your RNA of interest (include NM# and nucleotides covered if relevant)> by utilizing the Stellaris® RNA FISH Probe Designer (Biosearch Technologies, Inc., Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). The <samples> were hybridized with the <your RNA of interest> Stellaris FISH Probe set labeled with <your dye of choice> (Biosearch Technologies, Inc.), following the manufacturer’s instructions available online at www.biosearchtech.com/stellarisprotocols (access date). Briefly, <describe any deviations from the published protocol or a short summary of what was actually performed>.”

Citing Custom Probe Sets Utilizing Previously Published Sequences: “Custom Stellaris® RNA FISH Probes recognizing <your RNA of interest (include NM# and nucleotides covered if relevant)> and labeled with <your dye of choice>, were purchased from Biosearch Technologies, Inc. (Petaluma, CA). Probe set sequences utilized in the experiments have been previously described <cite published manuscript>. The <samples> were hybridized with the <your RNA of interest> Stellaris® RNA FISH Probe set, following the manufacturer’s instructions available online at www.biosearchtech.com/stellarisprotocols (access date). 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 Designed with the Stellaris® RNA FISH Probe Designer: “Custom 3' amine oligos in plates were designed against <your RNA of interest (include NM# and nucleotides covered if relevant)> by utilizing the Stellaris® RNA FISH Probe Designer (Biosearch Technologies, Inc., Petaluma, CA) available online at www.biosearchtech.com/stellarisdesigner (version #). Probes were labeled with <your dye of choice> using <insert your labeling protocol or citation of previously published labeling protocol>. The <samples> were hybridized with the <your RNA of interest> oligonucleotides (Biosearch Technologies, Inc.), following the manufacturer’s instructions available online at www.biosearchtech.com/stellarisprotocols (access date). 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 Utilizing Previously Published Sequences: “Custom 3' amine oligos in plates recognizing <your RNA of interest (include NM# and nucleotides covered if relevant)> were purchased from Biosearch Technologies, Inc. (Petaluma, CA). Probe set sequences utilized in the experiments have been previously described <cite published manuscript>. Probes were labeled with <your dye of choice> using <insert your labeling protocol or citation of previously published labeling protocol>. The <samples> were hybridized with the <your RNA of interest> oligonucleotides (Biosearch Technologies, Inc.), following the manufacturer’s instructions available online at www.biosearchtech.com/stellarisprotocols (access date). 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 e-mail our Technical Support Group at: techsupport@biosearchtech.com. Our knowledgeable staff is also available for telephone consultation from 8:00 AM to 5:00 PM, Monday through Friday, Pacific Time. Please contact us at:

1.800.GENOME.1 (436.6631) US & Canada only

+1.415.883.8400 telephone

+1.415.883.8488 fax

2199 S. McDowell Blvd, Petaluma, CA 94954-6904 USA | info@biosearchtech.com

©2015 Biosearch Technologies, Inc. All rights reserved.

This product is sold under license from Rutgers, The State University of New Jersey, and may be used under its patent rights for Research Use Only. Information on licensing programs for the commercial and/or diagnostic use of these products is available by contacting licensing@biosearchtech.com.

Biosearch, the Biosearch Technologies logo, and Stellaris are registered trademark of Biosearch Technologies, Inc., Products and technologies appearing in this brochure may have trademark or patent restrictions associated with them. Please see www.biosearchtech.com/legal for full legal disclosure.

UI-206656 Rev. 5.0
Eff. Date: 13 Apr 2015