Stellaris RNA FISH

Protocol for cells in suspension

General protocol and storage

Product description

A set of Stellaris™ RNA FISH Probes is comprised of up to 48 singly labelled 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.

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Stellaris RNA FISH

Protocol for cells in suspension

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) Deionised Formamide
f) Ethanol for molecular biology
g) Stellaris RNA FISH Hybridization 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) 18 × 18 mm square #1 coverglass
n) RNase free consumables such as pipette tips
o) Superfrost™ Plus Microscope slides
p) Kimwipes™
q) 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).

Stellaris RNA FISH

Protocol for cells in suspension

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 10 mL (100 mL), mix:

- 1 mL (10 mL) 37% Formaldehyde solution
- 1 mL (10 mL) 10X Phosphate Buffered Saline (PBS), RNase-free
- 8 mL (80 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.

Hybridization Buffer (1 mL/10 mL):

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 (10 mL) , mix:

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

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.

Stellaris RNA FISH

Protocol for cells in suspension

Wash Buffer A (10 mL/100 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 (100 mL), mix:

- 2 mL (20 mL) Stellaris RNA FISH Wash Buffer A
- Add 7 mL (70 mL) Nuclease-free water
- Add 1 mL (10 mL) Deionised Formamide
- 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) 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

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

Protocol for cells in suspension

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 Biology[3].

Fixation of cells in suspension

a) Centrifuge suspension cells (2 - 5 × 10^6 cells) in a 15 mL conical tube.
b) Aspirate supernatant, leaving cells in a pellet at base of tube.
c) Gently resuspend cells in 1 mL of 1X PBS, and centrifuge to pellet cell suspension.
d) Aspirate the 1X PBS, and gently resuspend cells in 1 mL of fixation buffer. Mix well by pipetting or inverting the tube.
e) Incubate at room temperature for 10 minutes.
f) 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.
g) To permeabilise 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 hybridisation.

Stellaris RNA FISH

Protocol for cells in suspension

Hybridisation of cells in suspension

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 e.

a) Invert tube with fixed and permeabilised 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 permeabilised suspension cells to a round #1 coverglass after which you can perform Stellaris RNA FISH following the protocol for adherent cells.
b) Centrifuge to pellet cells and aspirate 70% ethanol.
c) Gently resuspend cells in 500 µL of Wash Buffer A (see recipe above).
d) Centrifuge to pellet cells and aspirate Wash Buffer A.
e) Resuspend cells in 100 µL of Hybridization Buffer containing probe. Mix well by pipetting up and down.
f) Incubate microcentrifuge tube in the dark at 37 °C overnight (~16 hours).
g) 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.
h) Add 500 µL of Wash Buffer A. Centrifuge to pellet cells and aspirate solution. Be careful not to disturb the pellet.
i) Resuspend cells in 500 µL of Wash Buffer A.
j) Incubate in the dark at 37 °C for 30 minutes.
k) Centrifuge to pellet cells and aspirate Wash Buffer A.
l) Resuspend cells in 500 µL of DAPI nuclear stain (1X Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.
m) Incubate in the dark at 37 °C for 30 minutes.
n) Centrifuge to pellet cells and aspirate DAPI nuclear stain.
o) Resuspend cells in 500 µL of Wash Buffer B.
p) Centrifuge to pellet cells and aspirate Wash Buffer B. Resuspend cells in a small drop (approximately 30 µL) of Vectashield Mounting Medium.
q) Place 5-10 µL of cell suspension on a clean glass microscope slide and then place an 18 × 18 mm square #1 coverglass over the cells to spread the solution.
r) 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.
s) Seal the coverglass perimeter with CoverGrip (or clear nail polish), and allow to dry.

Proceed to imaging.

References

1. Raj, A., van den Bogaard, P., Rifkin, S.A., van Oudenaarden, A., and Tyagi, S. Imaging individual mRNA molecules using multiple singly labelled probes. Nat. Methods 2008; 5, 877-879. doi: 10.1038/nmeth.1253
2. 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
3. Dunagin, M., Cabili, M.N., Rinn, J., and Raj, A. Visualisation of lncRNA by single-molecule fluorescence *in situ* hybridisation. Nuclear Bodies and Noncoding RNAs: Methods and Protocols, Methods Mol. Biol. 2015; 1262, 3-19. doi: 10.1007/978-1-4939-2253-6_1

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>."

Stellaris RNA FISH

Protocol for cells in suspension

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.

For Research Use Only. Not for use in diagnostic procedures.

Integrated tools.
Accelerated science.

@LGCBiosearch | biosearchtech.com

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC