Stellaris RNA FISH

Protocol for *D. melanogaster* wing imaginal discs

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.

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Reagents and equipment

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)
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) 2-well chambered coverglass
m) 18 × 18 mm square #1 coverglass
n) RNase free consumables such as pipette tips
o) Humidified chamber (or equivalent): 150 mm tissue culture plate; place a single water-saturated paper towel 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 camera or sCMOS, 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 set volumes. 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 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 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
- 100 µL deionised 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
- Add 7 mL nuclease-free water
- Add 1 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 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 hybridisation in step j below.

Mounting media:

- Vectashield Mounting Medium

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

Protocol for *D. melanogaster* wing imaginal discs

NOTE: This protocol is adapted from the Raj lab protocol[3] and has not been tested at Biosearch Technologies. Currently, we can only offer limited support for the use of Stellaris probes on *D. melanogaster*.

Fixation of *D. melanogaster* wing imaginal discs using chambered coverglass

a) Submerge 3rd instar larvae in 1 mL of 1X PBS and dissect to release wing imaginal discs.
b) Place discs at the bottom of a chambered coverglass. The discs should readily stick.
c) Gently aspirate 1X PBS, and then add 1 mL of fixation buffer.
d) Incubate at room temperature for 45 minutes.
e) Aspirate fixation buffer, then wash twice with 1 mL of 1X PBS.
f) To permeabilise, aspirate 1X PBS and add 1 mL of 70% ethanol and store overnight at +2 to +8 °C. Discs can be stored at +2 to +8 °C in 70% ethanol up to a week before hybridisation.

Stellaris RNA FISH

Protocol for *D. melanogaster* wing imaginal discs

Hybridisation of *D. melanogaster* wing imaginal discs using chambered coverglass

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 d.

a) Aspirate the 70% ethanol off the chambered coverglass containing discs.
b) Add 1 mL of Wash Buffer A (see recipe above), and incubate at room temperature for 2-5 minutes.
c) Assemble humidified chamber: 150 mm tissue culture plate; place a single water-saturated paper towel alongside the inner chamber edge. This chamber will help prevent evaporation of the probe solution from under the coverglass.
d) Aspirate Wash Buffer A, and dispense 100 µL of the Hybridization Buffer containing probe onto the discs within the chambered coverglass.
e) Gently place a clean 18 × 18 mm #1 coverglass over the discs within the chambered coverglass.
f) Place the chambered coverglass into the humidified chamber. Cover the humidified chamber with the lid and seal with Parafilm®.
g) Incubate in the dark at 37 °C for at least 4 hours. (Incubation can be continued up to 16 hours).
h) Add 1 mL of Wash Buffer A to the chambered coverglass. Use forceps to carefully remove the 18 × 18 mm coverglass so as not to disturb the discs underneath.
i) Incubate in the dark at 37 °C for 30 minutes.
j) Aspirate Wash Buffer A, and then add 1 mL of DAPI nuclear stain (Wash Buffer A consisting of 5 ng/mL DAPI) to counterstain the nuclei.
k) Incubate in the dark at 37 °C for 30 minutes.
l) Aspirate the DAPI staining buffer, and then add 1 mL of Wash Buffer B. Incubate at room temperature for 2-5 minutes.
m) Add a small drop (approximately 15-30 µL) of Vectashield Mounting Medium onto the discs within the chambered coverglass. Place a clean 18 × 18 mm #1 coverglass over the discs to evenly spread the mounting medium.

Proceed to imaging.

Stellaris RNA FISH

Protocol for *D. melanogaster* wing imaginal discs

References

1. 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
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. Raj A, Tyagi S. Detection of individual endogenous RNA transcripts in situ using multiple singly labeled probes. Methods Enzymol. 2010; 472, 365-86. doi: 10.1016/S0076-6879(10)72004-8

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 <your dye of choice> (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 *D. melanogaster* wing imaginal discs

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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