Stellaris RNA FISH

Protocol for *D. melanogaster* embryo

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 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.

Embryo fixation solution

Embryo fixation solution should be stored at room temperature for short-term and long-term use.

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Embryo Wash Buffer

Embryo Wash Buffer should be stored at room temperature for short-term and long-term use.

50% bleach solution

Bleach solution should be stored at room temperature protected from light 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) Methanol for molecular biology
c) Sodium Hypochlorite
d) Sodium Chloride
e) Tween-20
f) 37% formaldehyde
g) Heptane
h) Ethanol for molecular biology
i) 10X Phosphate Buffered Saline (PBS), RNase-free
j) Nuclease-free water
k) Deionised formamide
l) Stellaris RNA FISH Hybridization Buffer (LGC, Biosearch Technologies Cat# SMF-HB1-10)
m) Stellaris RNA FISH Wash Buffer A (Biosearch Technologies Cat# SMF-WA1-60)
n) Stellaris RNA FISH Wash Buffer B (Biosearch Technologies Cat# SMF-WB1-20)
o) Prolong Diamond Antifade Mountant with DAPI (Life Technologies Cat# P36962 or equivalent)
p) 4',6-diamidino-2-phenylindole (DAPI)
q) Wheaton glass vials (Sigma Cat # Z115053 or equivalent)
r) #1 coverglass
s) Superfrost™ Plus Microscope slides
t) RNase free consumables such as pipette tips
u) 37 °C laboratory oven
v) Orbital platform shaker

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 (Xenon or LED are typically not bright enough).
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 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 250 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.*

50% bleach solution:

Create a 1:1 mix of nuclease-free water and Sodium Hypochlorite.

Embryo Wash Buffer*:

For a final volume of 1 L, mix:

- 1 L nuclease-free water
- 6 g Sodium Chloride
- 1 mL Tween-20 (Final concentration 0.1% v./v.).

* According to the European Union Chemical Agency's REACH Annex XIV certain detergents, including Triton X-100 and Tergitol (NP-40) are to be phased out. The Triton X-100 in older formulations of the wash buffer has been replaced with Tween-20. Other detergents proposed as Triton X-100-replacements include Zwittergen, Octyl glycoside, CHAPS and Nereid[7].

Embryo fixation solution:

For a final volume of 10 mL, mix:

- 0.5 mL nuclease-free water
- 0.5 mL 10X Phosphate Buffered Saline (PBS), RNase-free
- 4 mL 10% ultra-pure formaldehyde
- 5 mL Heptane.

PBT

Final composition is 0.1% (vol./vol.) Tween-20 in 1X PBS

For a final volume of 10 mL, mix:

- 10 mL 1X Phosphate Buffered Saline
- 10 µL Tween-20

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Hybridization Buffer (1 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, mix:

- 900 µL Stellaris RNA FISH Hybridization Buffer
- 100 µL 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. 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.

Wash Buffer A (10 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 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.

Mounting media:

- Prolong Diamond Mounting Medium

Protocol for *D. melanogaster* embryos

NOTE: This protocol has been adapted from a protocol provided by Dr. Matthew Ronshaugen and has not been tested at Biosearch Technologies. Currently, we can only offer limited support for the use of Stellaris probes on *D. melanogaster* embryos.

Protocol notes:

a) Glass Wheaton vials and glass pipettes should be used during steps involving Stellaris Wash Buffer A and Hybridization Buffer to prevent embryos sticking to the sides of plastic Eppendorf tubes.
b) Unless specified, use 0.5 mL volume per solution change.

Fixation of *D. melanogaster* embryos:

a) Collect eggs from healthy flies on apple juice agar plates smeared with yeast paste.
b) Dispense embryo wash buffer onto the agar surface, and gently brush the embryos off the agar with a paint brush and into a mesh basket. Wash away the yeast-filled suspension with embryo wash buffer.
c) Devitellinise the embryos in ~50% bleach for ~1-2 minutes, by periodically dispensing bleach onto the embryos and gently stir.
d) Wash thoroughly to remove all traces of bleach, alternating washes between double distilled water (ddH_2O) or nuclease-free water, which causes the embryos to clump together, and embryo wash buffer, which separates the clumps. Perform a final thorough wash in ddH_2O.
e) Using a paint brush, transfer embryos to a 20 mL scintillation vial containing 10 mL embryo fixation solution. The embryos should float at the interface between the two phases.
f) Cap the vial, tape it on its side to an orbital platform shaker and shake for 30 minutes at ~220 rpm.
g) After shaking, let the bubbles at the interface pop. Bubbles can be disrupted with a pipette tip. Completely remove the bottom, aqueous phase with a pipette, avoiding pulling up the embryos.
h) Add about 8 mL methanol, cap the vial and shake vigorously by hand for about 30 seconds, then place on the bench. The two phases will separate and the fixed, devitellinised embryos settle to the bottom in methanol. The upper phase of heptane should have remained, and all the burst vitelline membranes and non-devitellinised embryos will have remained in a cloudy layer at the interface.
i) First remove the heptane, then all the debris at the interface, then most of the methanol, leaving a few millimeters of methanol.
j) Rinse the embryos in fresh methanol, and using a pipette, transfer them in methanol to a 1.5 mL Eppendorf tube.
k) Wash the embryos with 3 changes (1 mL) of methanol. Embryos can then be stored in methanol at -20 °C for several years.

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Hybridisation of *D. melanogaster* embryos:

a) Aliquot ~50 µL embryos per staining scheme into glass Wheaton vials.
b) Make a transition from methanol to PBT: 25%, 50%, 100% (rocking at room temperature on a roller platform for 5 minutes each).
c) Rock 3x 10 minutes with PBT.
d) Rock in 50:50 PBT:Wash Buffer A, 10 minutes.
e) Rock 2x 5 minutes with Wash Buffer A.
f) Remove as much Wash Buffer A as possible, add 0.5 ml Hybridization Buffer to each vial, and allow embryos to settle for 5 minutes.
g) Replace with another 0.5 mL Hybridization Buffer, and incubate in a 37 °C water bath for 2 hours.
h) Prepare Stellaris probe mixtures, diluting probe to 50 nM in 0.25 mL Hybridization Buffer per vial.
i) Remove the Hybridization Buffer from embryos, and add 0.25 mL probe mixture to each vial.
j) Incubate in the dark in a 37 °C water bath for ~14 hours.
k) Remove probe mixtures, add 0.5 mL pre-warmed (37 °C) Hybridization Buffer, and incubate in the dark for 30 minutes at 37 °C.
l) Remove Hybridization Buffer, and wash with 0.5 mL pre-warmed (37 °C) Wash Buffer A.
m) Wash 3x 15 minutes with 0.5 mL pre-warmed (37 °C) Wash Buffer A in the dark at 37 °C.
n) Rock with 0.5 mL Wash Buffer A for 15 minutes in the dark at room temperature.
o) Rock 3x 10 minutes with PBT in the dark at room temperature.
p) Aliquot embryos to slides, and use an aspirator to thoroughly remove excess PBT, but without letting the embryos completely dry out.
q) Mount embryos under cover glass using Prolong Diamond Antifade with DAPI.
r) Dry slides flat in the dark at room temperature for 24 hours, then image immediately or store at -20 °C.

References

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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>."

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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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