Stellaris® RNA FISH Protocol for Frozen Tissue

LGC Biosearch Technologies

Abstract

A set of Stellaris FISH Probes comprises up to 48 singly labeled oligonucleotides designed to selectively bind to targeted transcripts. Stellaris FISH Probes bound to target RNA produce fluorescent signals that permit detection of single RNA molecules as diffraction-limited spots by conventional fluorescence microscopy. Please see the Biosearch

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Protocol

Fixation of frozen tissue sections

Step 1.
Frozen tissue must be sliced at a thickness of 4-10 µm using a cryostat and mounted onto a microscope slide.

Fixation of frozen tissue sections

Step 2.
Thaw the slide-mounted tissue section to room temperature.

Fixation of frozen tissue sections

Step 3.
Immerse the slide in fixation buffer for 10 minutes at room temperature.

DURATION
00:10:00

Fixation of frozen tissue sections

Step 4.
Wash with 1 mL of 1X PBS for 2-5 minutes.

AMOUNT
1 ml Additional info:
DURATION
00:05:00

Fixation of frozen tissue sections

Step 5.
Wash again with 1 mL of 1X PBS for 2-5 minutes.

AMOUNT
1 ml Additional info:
DURATION
00:05:00

Fixation of frozen tissue sections

Step 6.
To permeabilize the tissue section, immerse the slide in 70% ethanol for at least 1 hour at room

temperature.

DURATION

01:00:00

Hybridization in frozen tissue sections

Step 7.

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

Hybridization in frozen tissue sections

Step 8.

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, which is enough for one coverslip. This creates a working probe solution of 125 nM. This solution will be used in the steps below.

NOTES

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Formamide is a teratogen that is easily absorbed through the skin and should be used in a chemical fume hood.

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Be sure to let the formamide and Hybridization Buffer warm to room temperature before opening the bottle.

Hybridization in frozen tissue sections

Step 9.

Immerse the slide-mounted tissue section in 1X Wash Buffer 1 (see recipe on website) for 2-5 minutes.

DURATION

00:05:00

Hybridization in frozen tissue sections

Step 10.

Assemble humidified chamber: 150 mm tissue culture plate; a single water-saturated paper towel placed alongside the inner chamber edge.

NOTES

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This chamber will help prevent evaporation of the probe solution from the tissue section.

Hybridization in frozen tissue sections

Step 11.

Remove the slide from wash buffer, and carefully wipe away excess buffer surrounding the tissue section.

Hybridization in frozen tissue sections

Step 12.

Dispense 100 µL of Hybridization Buffer containing probe onto the tissue section of the slide.

AMOUNT

100 µl Additional info:

NOTES

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Note that 100 µL is recommended when using an 18 mm square coverglass, and if larger coverglasses are used, the volume will need to be adjusted accordingly

Hybridization in frozen tissue sections

Step 13.
Carefully place a clean 18 mm square coverglass over the Hybridization Buffer containing probe to completely cover the tissue section, and allow for even distribution of the solution. Place the slide in the humidified chamber, cover with the tissue culture lid, and seal with Parafilm.

Hybridization in frozen tissue sections

Step 14.
Incubate in the dark at 37 °C for at least 4 hours.

DURATION
04:00:00

NOTES
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Incubation can be continued up to 16 hours.

Hybridization in frozen tissue sections

Step 15.
Immerse the slide in Wash Buffer 1, and allow the submerged coverglass to slide off the tissue section.

NOTES
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Gentle agitation may be required to remove the coverglass.

Hybridization in frozen tissue sections

Step 16.
Incubate in the dark at 37 °C for 30 minutes.

DURATION
00:30:00

Hybridization in frozen tissue sections

Step 17.
Decant Wash Buffer 1, and then add DAPI nuclear stain (1X Wash Buffer 1 consisting of 5 ng/mL DAPI) to counterstain the nuclei.

Hybridization in frozen tissue sections

Step 18.
Incubate in the dark at 37 °C for 30 minutes.

DURATION
00:30:00

Hybridization in frozen tissue sections

Step 19.
Decant the DAPI staining buffer, and then immerse slide in Wash Buffer 2 for 2-5 minutes.

DURATION
00:05:00

Hybridization in frozen tissue sections

Step 20.
Remove the slide from Wash Buffer 2, and carefully wipe away excess buffer surrounding the tissue section.

Hybridization in frozen tissue sections

Step 21.
Add a small drop (approximately 15 µL) of Vectashield Mounting Medium onto the tissue section, and cover with a clean 18 mm square #1 coverglass.

AMOUNT

15 µl Additional info:

Hybridization in frozen tissue sections

Step 22.

Gently squeeze out excess anti-fade from underneath the coverglass.

Hybridization in frozen tissue sections

Step 23.

Seal the coverglass perimeter with clear nailpolish, and allow to dry.

Step 24.

Proceed to imaging.

Warnings

When performing Stellaris RNA FISH, it is imperative to limit RNA degradation. Please ensure that all consumables and reagents are RNase-free.