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RNA Fluorescent in situ hybridization (FISH) V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for RNA Fluorescent in situ hybridization (FISH)

Materials

REAGENT	SOURCE	IDENTIFIER
Formamide (Deionized)	Thermo Fisher Scientific	Cat#AM9342
RNAseZAP	Sigma Aldrich	Cat # R2020-250ML
ProLong Diamond Antifade mountant	Thermo Fisher Scientific	Cat #P36961
20xSSC	Thermo Fisher Scientific	Cat#AM9770
PBS 1x	Thermo Fisher Scientific	Cat#10010-023
Ultrapure Nuclease Free Water	Thermo Fisher Scientific	Cat#10977-015
38% Formaldehyde	Thermo Fisher Scientific	Cat# F79-1
200 Proof Ethanol (1 pint)	Decon laboratory	Cat# 2716
RNA-FISH probes	Biosearch Technologies	Probe sequence in excel file below

Materials:

1. Nuclease Free water (NF H_2O)
2. Formamide
3. 20X SSC and 2X SSC
5. PBS
9. 70% EtOH
10. Probe: 1 µL/reaction

* Probe prep: Stock ([M] 12.5 micromolar (µM) diluted in TE buffer)

SeV probes: 1:10 working solution (1.25uM) diluted in H_2O, 5 µL stock + 45 µL UltraPure H_2O, in vivo only

SeV probes: 1:100 working solution (125nM) diluted in H_2O, 5 µL 1:10 + 45 µL UltraPure H_2O, in vitro only

RSV probes: 1:50 working solution (250nM) diluted in H_2O, 10 µL 1:10 + 45 µL UltraPure H_2O, in vitro only

Solutions:

1. Fixation solution (4% FA):
   (prepare fresh) 5 mL 37% formaldehyde (100% formalin),
   45 mL PBS 1X
2. Wash buffer:
   (prepare fresh) 5 mL 20x SSC
   5 mL formamide

40 mL NF H_2O

3. 70% EtOH: 70 mL 100% EtOH,
30 mL NF H_2O

4. Hybridization buffer: 1 g dextran sulfate in 7 mL NF H_2O (mix by rotation, take half an hour to dissolve).
1 mL formamide
1 mL 20x SSC
Volume up to 10 mL
* Store at -20 °C in 500 µL aliquots; good for years.

5. Mounting media: ProLong Diamond Anti-fade Mountant (Cat #P36961)

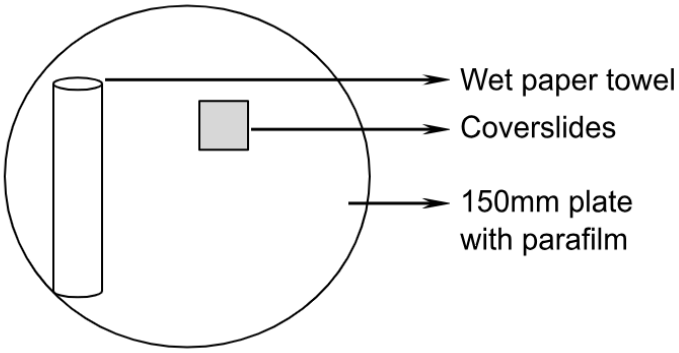
For GLOX anti-fade:

Anti-fade buffer: 850 µL Nuclease-Free H_2O
(per slide) 100 µL 20x SSC
40 µL 10% w/v glucose
10 µL Tris-HCl (pH 8)

Anti-fade solution: 100 µL Anti-fade buffer
1 µL glucose oxidase
1 µL catalase

Equipment:

1 Hybridization chamber:



How to mount the hybridization chamber.

Protocol:

2 **<u>Before starting, make sure to clean everything with RNaseZAP (Cat # R2020-250ML) and ethanol (i.e. gloves, pipettes, surfaces, etc) and always use filter tips.</u>**

Fixation and Permeabilization

1) Aspirate growth medium, and wash with 1 mL of 1X PBS.

2) Add 1 mL of fixation solution.

3) Incubate at room temperature for 10 minutes (you can move from tissue culture hood to your bench after this step).

4) Wash 2X with PBS.

5) Permeabilize cells in 1 mL of 70% ethanol for at least 1 hr at RT. Or O/N at 4 °C .

(Cells can be stored at 4 °C in 70% ethanol up to a week before hybridization).

Hybridization

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

— To prepare the hybridization solution, add 1 µL of probe working solution to 50 µL of hybridization buffer, and then vortex and centrifuge. *Final concentration of the probes should be tested and optimized.

1) Aspirate the 70% ethanol off the coverglass.

2) Add 1 mL of wash buffer, and incubate at RT for 2-5 minutes.

3) Prepare hybridization mix and assemble humidified hybridization chamber: see equipment

4) Add 50 µL of the hybridization buffer containing probe onto the parafilm.

5) Gently transfer the coverglass, cells side down, onto the hybridization solution. Cover the hybridization chamber (see equipment section) with the tissue culture lid. Incubate in the dark at 37 °C for at least 4 hours, better overnight.

Wash

1) Transfer the cover glass, cells side up, to a fresh plate containing 1 mL of wash buffer, immediately.

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

2) Wash with 1 mL wash buffer consisting of 5 ng/mL DAPI to counterstain the nuclei.

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

3) Wash with 1 mL of 2X SSC. Incubate at room temperature for 2-5 min.

1. 4) Mounting slides: Put a drop of ProLong Diamond Antifade (Cat # P36961) on to slide, dry off slide using aspirator and flip cell side down on to mounting media, make sure there are no bubbles. Mounting media needs to “cure” to correct refractive index overnight at room temerature (in dark), then can be moved to slidebox/proceed to imaging.

- Alternatively, GLOX anti-fade may be used if necessary/want to image right away. In this case, seal the coverglass perimeter with rubber cement, and allow to dry. Store slides on ice. If necessary, gently wipe away any dried salt off the coverglass with water. Proceed directly to imaging.

Lopez Lab Specifics

3 Probe preparation for hybridization:
When adding 1 µL of each probe in 50 µL of hybridization buffer, the final working probe solution should be 125 nM for SeV probes and 250 nM for RSV probes. Adjust depending on the probe set, virus and optimized dilution tested.
* It is always recommended to have immunofluorescence slides staining for viral protein as controls for efficient infection.

Protocol references

Updated by: Lavinia Gonzalez 03/31/23

Protocol adapted from BioreserachTech. For the original protocol:
https://www.biosearchtech.com/assets/bti_stellaris_protocol_adherent_cell.pdf