mono FISH on slides

Kat Dawson

Abstract

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Protocol

Preparation of cells on microscopic slide

Step 1.

We use PTFE coated slides that have 8-10 wells cut into the silicon. This way we can do a number of samples in the one hybridisation event without getting cross contamination of sample or probe.

HTC Autoclavable slides, 10 well 5 mm (Cel-line/Thermo Scientific 30-175)

Cover slips 24x60 mm (VWR 48393-106)

1. Spot 2-5 µl of pfa fixed cells onto slide and allow to air dry.
2. Dehydrate by immersing slide for 2 minutes each in 50%, then 80% and finally 90% ethanol.
3. Slide can now be hybridized for FISH or stored for later use.

Hybridization on slides

Step 2.

1 M Tris/HCl pH 8.0

5 M NaCl

10% SDS

Formamide

(*Filter sterilize all solutions*)

Fluorescent labelled probe, at 25-50 ng/µl

50 ml tube with kim wipe

Hybridization oven at 46 ˚C

Water bath at 48 ˚C

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Vectashield (Vector labs H-1000)

1. Prepare a slide with pfa fixed cells. (It's good practice to include positive and negative controls for the probes to be used when possible).
2. Make 2 ml of hybridization buffer at desired stringency (see table below). Add in this order: water, NaCl, Tris, SDS, formamide. Hybridization buffer can be stored at -20 to be used in future experiments.
3. Place a kim wipe in a 50 ml tube and add about 1.8 ml of hybridization buffer. Allow to equilibrate in the oven.
4. If two probes are to be used, add 8 µl of hybridization buffer to each well. If three probes are to be used, add 7 µl.
5. Add 1 µl of each probe working stock (50 ng/µl) to have a final probe concentration of 5ng/µl.
6. Place slide in the 50 ml tube, being sure to keep it level, and incubate at 46°C for 2 to 24 hours.
7. While waiting, make wash buffer with an appropriate NaCl concentration (see table below). Pre-incubate this at 48°C. Wash buffer should be made fresh each time.

Wash ste

Step 3.

1. Remove slide from the oven and rinse with a small amount of the wash buffer before fully immersing in the wash buffer for 15 min at 48°C.
2. Rinse by immersing slide in DI water.
3. Shake off excess water and allow the slide to dry.
4. After drying, the slide can be counter-stained with DAPI.

Counter-stainging with DAPI

Step 4.

4',6-diamidino-2-phenylindole (DAPI) (Hicks *et al.*, 1992) is a fluorescent stain that strongly binds DNA. It passes through the cell membrane and will stain both live and fixed cells. The absorption maximum is 358 nm and the emission maximum is 461 nm (blue/cyan).

1. Following FISH or to visualize cells spotted on a slide add 10 µl of DAPI solution (25ng/µl in water).
2. Incubate at room temperature for 5 min.
3. Wash with DI water and let the slide dry
4. Mount cover slip using several drops of vectashield pressing firmly to squeeze out excess mounting solution.

Slides are best viewed immediately, but can be stored for several days to months at -20 °C with minimal fading.

Hyrbridization Buffer T

Step 5.

To a 2 ml tube add:

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- 40 µl 1M TRIS/HCl pH 8.0

- 2 µl 10% SDS

- 360 µl 5M NaCl

- x µl formamide

- y µl MQ water

To determine (x) the amount of formamide:

Formamide %	Formamide (x) µl	MQ water (y) µl
0%	0	1598
5%	100	1498
10%	200	1398
15%	300	1298
20%	400	1198
25%	500	1098
30%	600	998
35%	700	898
40%	800	798
45%	900	698
50%	1000	598
55%	1100	498
60%	1200	398

Wash Buffer Table

Step 6.

Prepare in a 50 ml Falcon tube:

To about 40 ml of MQ water add

- 50 µl 10% SDS

- 1 ml 1M TRIS/HCl ph 8.0

- z µl 5M NaCl (see table below)

- make up to 50 ml with MQ water

Hybridisation stringency % Formamide	NaCl molar	Volume of 5M NaCl to add to wash buffer (z µl)

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0%	0.900	9000
5%	0.636	6300
10%	0.450	4500
15%	0.318	3180
20%	0.225	2150
25%	0.159	1490
30%	0.112	1020
35%	0.080	700
40%	0.056	460
45%	0.040	300
50%	0.028	180
55%	0.010	100
60%	0.004	40

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