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Solutions used for FISH Codetection

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We use this protocol and it's working

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Abstract

protocol to make the solutions used in the protocol published by AWM lab for FISH Codetection

Troubleshooting



WATER

- 1 -1- MilliQ water directly pick up at the source...
-2- nuclease free water (Invitrogen AM9932)

PFA pH 7,5

- 2 40g in 900 ml H₂O + 1 drop 10N NaOH
Heat for dissolution (no more than 65°C)
Then add 100 ml of x10 PBS
Filter 0,2 µm
Cool on ice for an immediate use.
Could be kept at 4°C 15 days without problem or at -20°C

TEA pH 8 (keep at RT)

- 3 13,3 ml TEA (MERCK)
Qsp 1 litre H₂O
Adjust to pH=8.0 (with conc HCl)

HybBuffer: Hybridization buffer

- 4 Hyb Buffer without formamide 2 ml
 - 20X SSC 10ml
 - E.coli tRNA 50mg/ml 200 µl
 - RNase free water 4 ml
 - X50 Denhardt's 4ml
 - Salmon Sperm DNA 10 mg/ml 2 mlAliquote in 2ml tube. Keep aliquots at -20°C.
These could be thawed and refreezed without pb!

Desionized Formamide

- 5 Important to use desionized formamide of good quality.
For this, formamide must be desionized in the lab, aliquoted (in 600 µl tube (500 µl/tube) or 1,5 ml tube (1 ml/tube) and aliquots kept at -80°C and not at -20°C! If you thaw a tube **you should not refreeze it.**



To deionize 500 ml formamide

1. Weigh 25g resin AG501-X8
2. Add 500 ml formamide (pH 7).
3. Stir for 2 h.
4. Verify pH to 5.5 (if the pH is still 7, discard the solution completely).
5. Filter the solution with 0,22 µm nylon filter (do not use nitrocellulose filter!)
6. Label the tubes with the content.

Freeze aliquots to not be used immediately.

5X MAB (stock solution: keep at RT)

- 6 Maleic Acid: 58,04 g
NaCl : 43,82 g
Dilute in 850 ml H₂O and adjust the pH to 7,5 using 10N (30%) NaOH
(for this add 90ml of NaOH then drop by drop. Note that the solution may stay turbid until the pH reach around 6,8).
Then qsp 1 liter with H₂O

MABT

- 7 MAB + 0,1% Tween20Untitled section

PBST

- 8 PBS + 0,1% Tween 20

BR (Blocking Reagent Buffer): keep at -20°C

- 9 For 200 ml
5x MAB : 40ml
FBS heat inactivated:40 ml
Blocking Reagent (Roche) 2 g
Qsp 200 ml H₂O
Make the solution and aliquote it in 15 ml tube. Could be thawed and refreezed.

Fluorescein-Tyramide

- 10 - Solution 1 : Make a 10 mg/ml stock of the NHS ester in DMF (100 mg fluorescein NHS ester in 10 ml DMF)



- Solution 2 : Make DMF-TEA solution (4 ml DMF (dimethylformamide) + 40 ul TEA (triethylamine))
- Solution 3 : Make Tyramide solution (35 mg tyramide in 3,5 ml DMF-TEA (ie in solution 2))

To link the Fluorescein onto tyramide, the reaction is :

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Mix 10 ml fluorescein-NHS in DMF (ie solution 1) + 3,43 ml tyramide solution (ie solution 3)

- Incubate in dark at room temp for 2 hours
- Then add 12 ml ethanol,
- Make aliquots of 500µl in 600µl tube store in dark at 4 or -20°C

Glycine 1M Ph2,1

11 For 100 ml:

- 7,5 g Glycine
- Add 90 mlH₂O
- Adjust the pH to 2,1
- Qsp 100 ml H₂O
- Aliquot in 10 ml tube

Keep aliquots at -20°C.

Dilute 10x in PBS before use.

DAPI

12 Stock: 5 ng/ml in water (this is kept in minus 20)
Dilute 1:25.000 in PBST (2 µl per 50 ml), keep at 4°C