FISH Probes – Automated Hybridization Protocol

Notes

- Protocol can be used with all FISH probes – controls, gene specifics, custom FISH probes.
- Solutions can be made prior to the procedure.
- Further optimization of the protocol may be required.

Required Reagents & Equipment (Not Supplied)

HYBrite or ThermoBrite
Absorbent material
dH_2O
Wash Solution 1 (WS1) – 0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC
Wash Solution 2 (WS2) – 0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC
DAPI with Antifade

Automated HYBrite / ThermoBrite Protocol

1. Turn on HYBrite / ThermoBrite.
2. Set program. Please see guide below.
3. Presoak absorbent material in dH_2O and position in HYBrite / ThermoBrite.
4. Add 10 µl probe mixture to slide (2 µl probe + 8 µl hybridization buffer, or for multiple individually supplied probes per slide use 2 µl of each probe plus hybridization buffer for a total volume of 10 µl per slide).
5. Apply clean 22 mm^2 coverslip to slide.
6. Apply rubber cement on edges of coverslip to seal.
7. Place in HYBrite or ThermoBrite and close lid.
8. Start program. Hybridization will take at least 16 hours. See below for program recommendations.
9. Pre-warm WS1 (0.3% Igepal (Sigma CA-630) or NP-40 / 0.4 x SSC) to 73°C.
10. Remove coverslip. Place in WS1, agitating for approximately 10 seconds then let stand for exactly 2 minutes.
11. Transfer to WS2 (0.1% Igepal (Sigma CA-630) or NP-40 / 2 x SSC) at room temperature for 1 minute.
12. Let dry in dark.
13. Apply 10 µl DAPI with Antifade and 22 mm^2 coverslip.
14. Wait 15-30 minutes then visualize under microscope using the appropriate filter sets.

HYBrite / ThermoBrite Program Guide

Peripheral Blood Preparations
Denature at 72-73°C for 2 minutes. Hybridize at 37°C for at least 16 hours.

Paraffin Embedded Tissue Sections (after pretreatment)
Denature at 83°C for 3 minutes. Hybridize at 37°C for at least 16 hours. May require troubleshooting.

Recommendations

Optional FISH Pretreatment
We recommend the Abbott Molecular FISH Pretreatment Reagent Kit.

Paraffin Pretreatment
Please see the Empire Genomics Paraffin Embedded Tissue Sample Slide Processing Protocol.
Alternatively we recommend the Abbott Molecular Paraffin Pretreatment Reagent Kits: I, II or III.

References

Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual, Third Edition. Lippincott-Raven Philadelphia. 1991.

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Dye Specification Sheet

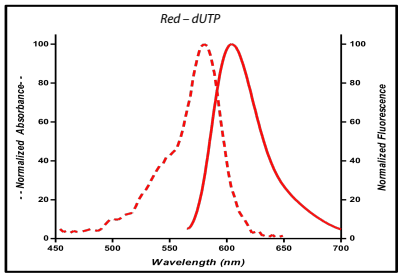
BAC DNA Library	RP11®, RP23®				
Cot-1 DNA	15 µg (3 µg/reaction)				
Diluent	1x Hyb Buffer				
Label	Red-dUTP	Green-dUTP	Orange-dUTP	Gold-dUTP	Aqua-dUTP
Fluorophore	5-ROX (5-Carboxyl-x-rhodamine)	5-Fluorescein	5-TAMRA	Carboxyrhodamine 6G	Aqua
Color	Red	Green	Orange	Gold	Aqua
Absorbance Maximum	580 nm	491 nm	548 nm	525 nm	418 nm
Emission Maximum	599 nm	515 nm	573 nm	551 nm	467 nm

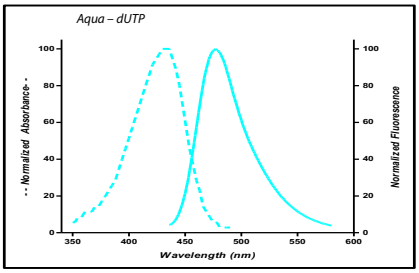
INSTRUCTIONS

- Gently vortex and centrifuge tube prior to use
- Store at -20˚C in a manual defrost freezer
- Protect from light
- Minimize freeze-thaw cycles

FISH PROTOCOL

Please refer to the FISH & Hybridization Protocol on our website:
http://www.empiregenomics.com/docs/HybridizationQuickReference-EmpireGenomics.pdf





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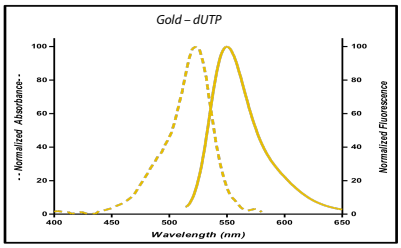

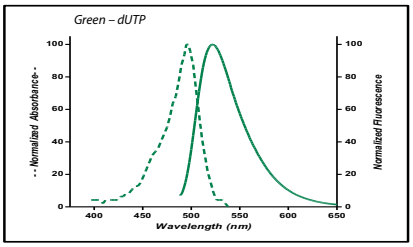

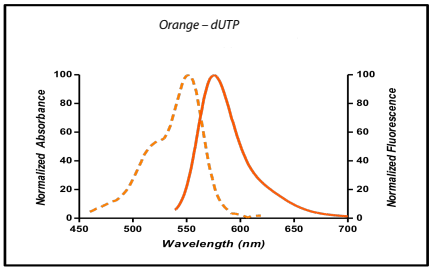

* Please Note: The human eye visualizes the Aqua wavelength more poorly than other regions of the visible light spectrum (as above). Consequently, when choosing to use an aqua probe, it is best to use it with a target that hybridizes strongly. For example, in our own experiences we have had better success with centromere probes compared to locus probes. Our Aqua probes have been benchmarked against the leading competitors and we are as bright as or brighter than they are. This material has passed our Quality Control processes and meets performance benchmarks. We offer a variety of colors for FISH probe labeling and if you want a probe with a stronger signal we would suggest you consider using green, gold, orange or red ones. We cannot guarantee the performance you will experience with the aqua dye as a results of the many variables which can affect its performance

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