EXIQON
Seek Find Verify

In situ hybridization on EDC-fixed FFPE tissues using DIG-labeled miRCURY LNA™ detection probes

By Dr. Liming Luan, the Thomas Andl Laboratory, Vanderbilt University, Nashville, USA.

Deparaffinize the sections

1. Xylene, 3 X 5 min.
2. Ethanol, 100% 2 X 5 min.
3. Ethanol, 70% 5 min.
4. Ethanol, 50% 5 min.
5. Ethanol, 25% 5 min.
6. RNase-free H_2O, 1 min.

Deproteinate the sections

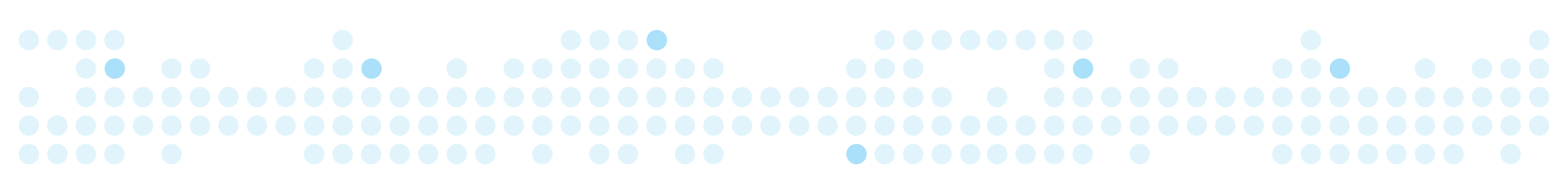
7. Wash slides with PBS, 2 X 5 min.
8. Soak slides in 75 ml PBS containing 20 µg/ml Prot. K (P4850, Sigma), 20 min.
9. Wash slides with PBS, 2 X 5 min.
10. Fix sections with fresh 4% PFA (in PBS), 10 min.
11. Wash slides with 0.2% Glycine (in PBS), 5 min.
12. Wash slides with PBS, 2 X 5 min.

EDC fixation

13. Incubate slides in 75 ml of freshly prepared imidazole buffer, 2 X 10 min.
14. Circle the sections with a DAKO pen.
15. Place slides in a humidified chamber (dH2O), add 500 µl freshly prepared EDC solution to each slide and incubate for 1-2 h at room temperature.
16. Wash slides with 0.2% Glycine (in PBS), 5 min.
17. Wash slides with PBS, 2 X 5 min.

Prehybridization and hybridization

18. Add 500 µl prehybridization buffer (50% Formamide, 5X SSC) to each slide, room temperature, 15 min.
19. Denature the probe at 85°C for 3 min, chill on ice.
20. Add probe (final concentration, 20 nM) in hybridization buffer (50% Formamide, 5X SSC, 10% Dextron Sulphate Sodium (DSS), 500 µg tRNA, 0.02% BSA).
21. Add 200 µl hybridization mix per slide and cover with baked cover glasses.
22. Hybridize sections in a humidified chamber (50% Formamide, 5X SSC) for 16-18 hours.
    Hybridization temperature = DNA *T*m probe - 21°C.



Stringency Wash

23. Wash slides in 5X SSC, 50% Formamide at hybridization temperature, 15 min.
24. Wash slides in 2X SSC, 50% Formamide at hybridization temperature, 30 min.
25. Wash slides in 0.2X SSC, 50% Formamide at hybridization temperature, 2 x 30 min.
26. Rinse slides in 0.2X SSC at room temperature, 5 min.

Immunological Detection

27. Soak slides in Buffer I [150 mM NaCl, 100 mM Tris-Cl (pH7.5)] at room temperature for 5 min.
28. Add 200 µl of blocking buffer [1% Blocking reagent (Roche) in Buffer I] to each slide, room temperature, 1 hr.
29. Antibody incubation [anti-DIG-AP Fab fragment (Roche),1:5000, in blocking buffer], 4°C, overnight.
30. Wash slides in Buffer I, 3 x 5 min.
31. Wash slides in Buffer II [100 mM NaCl, 100 mM Tris-Cl (pH9.5)], 5 min.

Color Development

32. Mix 200 µl of the NBT/BCIP Stock Solution (Roche) with 10 ml Buffer II.
33. Add diluted NBT/BCIP Solution to the slides and keep them in a light-protected humidified box, room temperature, 1-4 hr. For slow color development, transfer the box to 4°C, overnight.
34. Wash slides with PBS, 3 x 5 min.
35. Wash slides with dH_2O, 5 min.
36. Counter stain sections with Methyl green.

Reagents and Buffers

Imidazole buffer (160 ml, 0.13 M 1–methylimidazole, 300 mM NaCl, pH 8.0)
Add 1.6 ml of 1-methylimidazole to 130 ml water, adjust the pH by adding approximately 450 µl 12 M HCl to pH 8.0, then add 16 ml 3 M NaCl and water to a final volume of 160 ml.

EDC solution (0.16 M l–ethyl–3– (3–dimethylaminopropyl) carbodiimide (EDC))
Add 176 µl EDC into 10 ml of imidazole buffer, and then readjust the pH of the EDC solution by adding about 100 µl 12 M HCl to pH 8.0.

References

1. http://www.exiqon.com/ls/Documents/Scientific/FFPE%20in%20situ%20hybridization.pdf
2. Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. 3rd ed.New York: CSHL, 2001.
3. Pena *et al.* miRNA *in situ* hybridization in formaldehyde and EDC-fixed tissues. Nat. Methods. 2009, 6: 139-41.

DIG: DIG is licensed from Roche Diagnostics GmbH.

"This protocol has been developed by a third party and not by Exiqon A/S or group companies ("EXIQON").

Thus, EXIQON cannot and will not warrant, represent or in any other way guarantee that the protocol and its content comply with your needs or expectations. EXIQON excludes all liability for any illegality arising from error, omission or inaccuracy in the protocol and takes no responsibility for the protocol or otherwise.

To the extent permitted by law, EXIQON excludes all liability in contract, tort (including negligence), breach of statutory duty or otherwise for any costs, losses, claims, damages, expenses or proceedings (including special, incidental or consequential loss or damage, loss of profits and wasted management time) incurred or suffered by you arising directly or indirectly in connection with the protocol and its content, including any loss, damage or expense arising from, but not limited to, any defect, error, imperfection, fault, mistake or inaccuracy with the protocol and its content. Any dispute relating to the protocol involving EXIQON shall be governed by Danish law."