PRECISIONARY

IN-SITU HYBRIDIZATION

PROTOCOL FOR IN-SITU HYBRIDIZATION (DNA & RNA)

Key to reading the protocol:

√ Rationale for procedural step

♠ Tips & Tricks

1. For in-situ hybridization (ISH), fresh tissue should be fixed in 4% paraformaldehyde overnight, then rinsed in 1X PBS the next day. Tissue should be sectioned with the Compresstome® for desired slices.
   - √ Be sure to not "over-fix" tissue in paraformaldehyde! Doing so results in excessive cross-linking of proteins that can interfere with getting good signals for in-situ hybridization.
   - ♠ What slice thickness should you aim for? For ISH, slices should be 3 µm to 7 µm thick. They can be as thick as 10 µm.
2. Mount free-floating slices onto Superfrost-Plus slides (Fisher Scientific).
   - √ Why use Superfrost-PLUS slides? Because there is a (+) charge on these types of slides and allow tissue slices to adhere to the slide surface. Therefore, when you are processing the slides, the slices won't just fall off.
3. Dry slices on slide using a slide warmer for 30 min at 37°C.
4. Fix slides again in 4% paraformaldehy in diethyl pyrocarbonate (DEPC) H_2O for 20 min.
5. Rinse slides with DEPC PBS (2 x 5 min).
6. Deproteinate slices with proteinase K treatment (50 µg.ml; 1M Tris HCl at pH 7.5, and 0.1M EDTA) for 30 min at 25°C.
7. After deproteination, rinse sections in DEPC PBS (2 x 5 min).
8. Fixed slides in 4% paraformaldehyde in DEPC H_2O for 20 min.
9. Briefly rinse slides in DEPC H_2O.

10. Next, hybridize slices in hybridization buffer containing the oligonucleotides you are using.

    Incubate for 24 hours at 37°C in a humid chamber.
11. After hybridization, wash sections in 0.2X SSC (4 X 20 min each) at 47°C.
12. Block slices in blocking protein in Tris buffered saline (TBS) for 30 min (Perkin Elmer Amplification

    Kit).
13. Incubate slides in digitoxigenin-horseradish peroxidase in TBS containing Tween 20 (TBST; 8

    µl/ml) for 30 min at 25°C.
14. Rinse slides in TBST (3 X 5 min).
15. Incubate slides in tyramide solution (1:50 in kit diluent) for 10 min at 25°C.
16. Rinse slides in TBST (3 X 5 min).
17. Incubate slides in streptavidin-F (1:50) in TNB buffer (0.05 g/ml block protein in Tris-saline) for 30

    min.
18. Rinse slides in TBST (3 X 5 min).
19. Air dry slides and mount coverslips using Vectashield Mounting Media.

References

*** Uses the Compresstome® for successful in-situ hybridization experiments.**

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