ImmunoFISH for Mice and Baboons Frozen Sections

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[Abstract] This protocol is optimized for immunoFISH staining of OCT section of mouse tissues. It combines immunofluorescence for DNA damage response factors (*e.g.* 53BP1) (Le *et al.*, 2010) and FISH against telomeric DNA.

Materials and Reagents

1. Tissue
2. OCT
3. 4% formaldehyde
4. PBS
5. Triton
6. Goat serum
7. BSA
8. Primary antibody: 53BP1 #NB 100-304 rabbit from Novus
9. Second antibody: goat anti-rabbit Alexa Fluor® 488 Dye
10. Triton
11. Glycine
12. Mowiol 4-88 reagent (Calbiochem®)
13. Formamide
14. Tris HCl, pH 7.4
15. Telomeric PNA probe (TelC-Cy3 from PANAGENE, catalog number: F1002-5)
16. Tween-20
17. DAPI
18. Hybridization mixture (see Recipes)
19. Blocking reagent (Roche Diagnostics, catalog number: 11096176001) (see Recipes)
20. Wash solution I (see Recipes)
21. Wash solution II (see Recipes)

Equipment

1. Glass slide
2. Metal thermoblock
3. Humidified chamber

Procedure

1. Frozen tissue placed in OCT without fixation.
2. When needed, slice to the desired thickness (8-10 micron), dry the slides few minutes (often the time to prepare the other slides) and freeze again at -80 °C.
3. The day of the staining, thaw the slides and fix for 20 min in 4% formaldehyde.
4. Wash slides with PBS for 3 x 5 min at RT.
5. Permeabilize slides with 0.5% Triton in PBS for 5 min at RT.
6. Wash 2x with PBS 5 min at RT.
7. Block in 5% Goat serum diluted in PBS + 1% BSA for 60 min.
8. Incubate at 4 °C: 53BP1 #NB 100-304 (rabbit from Novus) 1:100 in PBS, 2.5% goat serum, 1% BSA. Use 60-80 µl for each slide.
9. Wash once quickly and 3 x 10 min with PBS at RT.
10. Secondary: goat anti-rabbit (Alexa 488) (1/100) in PBS + 1% BSA for 60 min at RT.
11. Wash once quickly and 3 x 10 min with PBS RT.
12. Re-fix tissue with PFA 4% + Triton 0.1%, 10 min RT.
13. Incubate with glycine 10 mM in H_2O, 30 min, RT.
14. Wash with 1x PBS, 3 times, 5 min.
15. Prepare the hybridization mixture and put 30-50 µl directly on the sample.
16. Put a glass slide carefully on the drop without making bubbles.
17. Put the slide directly on a metal thermoblock at 80 °C, 5 min.
18. Hybridize in a humidified chamber, 2 h, RT.
19. Remove glass from the slide.
20. Wash with Wash solution I, twice, 15 min.
21. Wash with Wash solution II, 3 times, 5 min.
22. Incubate with DAPI, 2 min, RT.
23. Wash briefly with 1x PBS.
24. Mount with mowiol.
25. Store the slides at 4 °C for short time storage (2 weeks) or at -20 °C. It is recommended to analyze the fluorescence as soon as possible to avoid fluorophore fading.

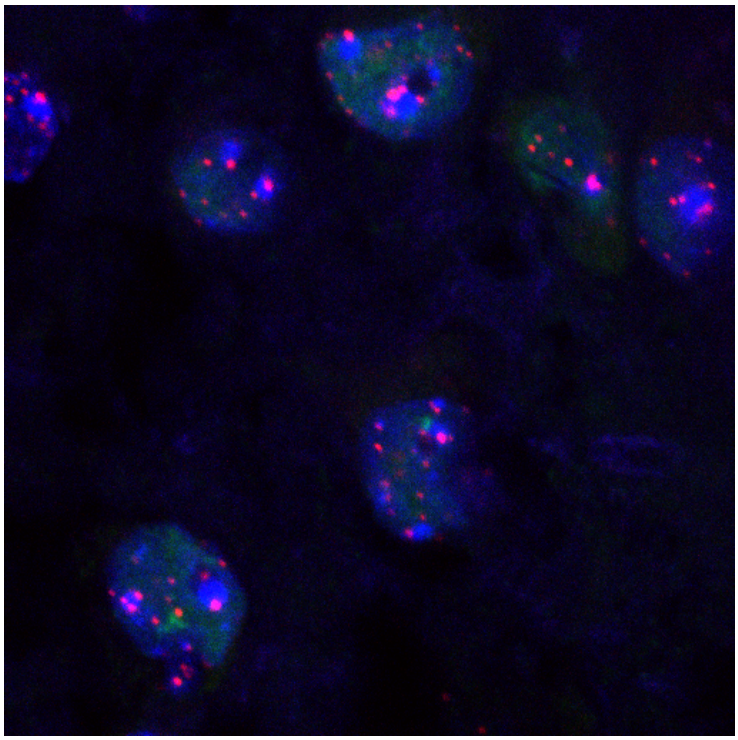

Figure 1: A representative figure of ImmunoFISH stained mouse hippocampus tissue. DAPI is in blue, 53BP1is in green and telomeric PNA probe is in red.

Recipes

1. Hybridization mixture (always prepare fresh)

Formamide	70%
Blocking reagent	1x
Tris HCl (pH 7.4)	10 mM
Telomeric PNA probe	0.5 µM
H_2O	to volume

2. 10x Blocking reagent

   Prepare small aliquots and store them at -20 °C.

3. Wash Solution I (250 ml) (always prepare fresh)

Formamide	175 ml
BSA 10%	2.5 ml
Tris HCl 1 M pH 7.4	2.5 ml
H_2O	to volume

4. Wash Solution II (350 ml) (always prepare fresh)

Tris HCl 1 M pH 7.4	35 ml
NaCl 5 M	10.5 ml

Tween-20 10%	2.5 ml
H_2O	to volume

Acknowledgments

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