ImmunoFISH for Adherent Cultured Mammalian Cells

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[Abstract] This protocol is optimized for immunoFISH staining of adherent cultured mammalian cells. It combines immunofluorescence for DNA damage response factors (*e.g.* 53BP1) and FISH against telomeric DNA.

Materials and Reagents

1. Cells
2. 4% PFA
3. Methanol/acetone 1:1
4. TritonX100
5. Primary antibody : 53BP1 #NB 100-304 rabbit from Novus
6. Second antibody: goat anti-rabbit Alexa Fluor® 488 Dye
7. PBS
8. Glycine
9. Fish gelatin (Sigma-Aldrich, catalog number : G7041)
10. BSA
11. Formamide
12. Tris HCl, pH 7.4
13. Telomeric PNA probe (TelC-Cy3 from PANAGENE, catalog number: F1002-5)
14. DAPI
15. Mowiol 4-88 reagent (Calbiochem®)
16. PBG (see Recipes)
17. Hybridization mixture (see Recipes)
18. Blocking reagent (Roche Diagnostics, catalog number: 11096176001) (see Recipes)
19. Wash solution I (see Recipes)
20. Wash solution II (see Recipes)

Equipment

1. Glass coverslips

2. 12 multiwell plate
3. Metal thermoblock
4. Humidified chamber

Procedure

1. Grow cells on glass coverslips (*e.g.* BJ normal human fibroblasts).
2. Transfer the coverslip to a 12 multiwell plate.
3. Wash briefly with 1x PBS.
4. Fix with either 4% PFA, 10 min, RT or methanol/acetone 1:1, 2 min, RT (it depends on the antibody, does not affect the FISH signal; use methanol/acetone for 53BP1 staining).
5. Wash with 1x PBS, 3 times, 5 min.
6. Only for PFA-fixed cells, incubate with 0.2% TritonX100 in PBS, 10 min, then wash with 1x PBS, 3 times, 5 min.
7. Block with 1x PBG, 1 h, RT.
8. Incubate with primary antibody diluted in 1x PBG, 50 µl for each coverslip. Incubation time depends on the antibody, most work in 1 h, RT, or overnight at 4 °C. (For 53BP1 dilute 1:200 and incubate 1 h at RT).
9. Wash with 1x PBG, 3 times, 5 min.
10. Incubate with secondary antibody diluted in 1x PBG, 45 min, RT.
11. Wash with 1x PBG, twice, 5 min.
12. Wash with 1x PBS, twice, 5 min.
13. Re-fix cells with PFA 4% + triton 0.1%, 10 min RT (use PFA also if you have previously fixed cells with methanol/acetone).
14. Incubate with glycine 10 mM in H_2O, 30 min, RT.
15. Wash with 1x PBS, 3 times, 5 min.
16. Prepare the hybridization mixture and put 20 µl on a glass slide for each coverslip.
17. Transfer the coverslip carefully on the drop without making bubbles.
18. Put the slide directly on a metal thermo block at 80 °C, 5 min.
19. Hybridize in a humidified chamber, 2 h, RT.
20. Remove coverslip from the slide and put it back in the 12 wells plate.
21. Wash with Wash solution I, twice, 15 min.
22. Wash with Wash solution II, 3 times, 5 min.
23. Incubate with DAPI, 2 min, RT.
24. Wash briefly with 1x PBS.
25. Mount with mowiol.

26. 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.

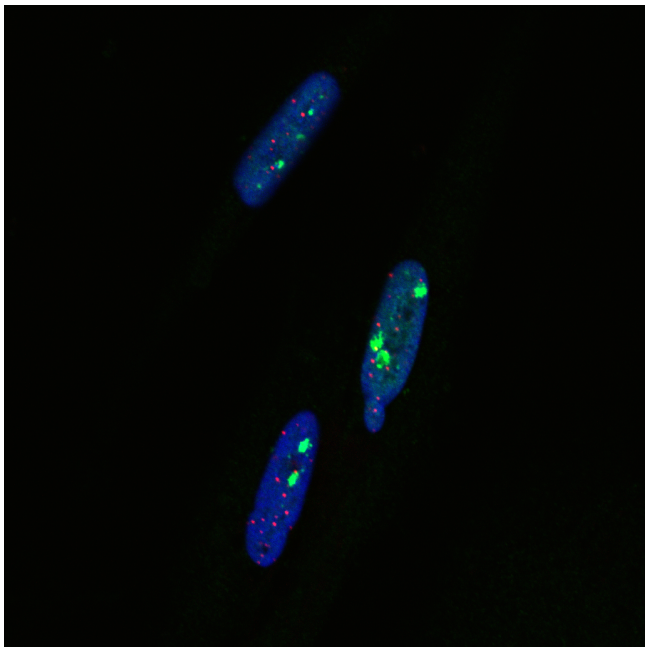

Figure 1. An image of ImmunoFish stained human fibroblasts cells. DAPI is in blue, 53BP1is in green and telomeric PNA probe is in red.

Recipes

1. 10x PBG (prepare 5 ml aliquotes and store them in 50 ml tubes at -20 °C, the day of immunoFISH dilute them in 1x PBS)

Fish gelatin	2%
BSA	5%
1x PBS	to volume

2. 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

3. 10x Blocking reagent

   Prepare small aliquots and store them at -20 °C

4. 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

5. 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

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References

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