Immunofluorescence to monitor APBs (ALT-associated PML Bodies)

To detect APBs we perform double labeling IF experiments with antibodies recognizing telomeres (either TRF1 or TRF2) and an antibody recognizing the protein PML.
Culture cells in chamber slides until approximately 70% confluence.
Wash once with ice-cold PBS + 0.2% Tween.
Pre-extraction:
Put slides on ice, then add ice-cold 0.5% Triton X-100 in PBS to cells, and keep for 1 min.
Wash once with ice-cold PBS + 0.2% Tween immediately.
Fixation and permeabilization:
Fix cells with 4% paraformaldehyde in PBS for 10 min at RT.
Permeabilize cells with methanol:acetone (1:1) at -20oC for 15 min.
Wash 3 X in ice-cold PBS + 0.2% Tween.
Incubation with antibodies:
Block cells with 10% Goat serum in PBS + 0.2% Tween for 15 min at RT.
Incubate cells with primary antibodies (PML and TRF1) diluted in blocking buffer for 1 h at RT (covered) or O/N at 4oC.
Wash at least 3 X 5 min in PBS + 0.2% Tween.
Incubate with secondary antibodies diluted in blocking buffer for 60 min at RT.
Wash 3 X 5 min in PBS + 0.2% Tween.
Cover slides with mounting media with DAPI and seal.

Immuno-FISH to monitor APBs (ALT-associated PML Bodies)

When the labeling with antibodies against TRF1 or TRF2 is not good, we perform Immuno-FISH. That is, we do IF for PML followed by FISH with a telomeric probe (PNA probe). However, the only PML Ab that works well for Immuno-FISH is the one by Paul Freemont (Imperial College London).
The procedure is the same described above, except that the cells are only incubated with anti-PML antibody and the appropriate secondary antibody (not Cy3, best FITC or Alexa conjugated).

Remove chambers from the slides:
After washing 3 X 5 min in PBS + 0.2% Tween, chamber slides are removed and slides are placed in glass staining dishes. During all the incubations the glass staining dishes containing the slides are rocked slowly in a rocking platform.
Fixation and hybridization:
Wash slides 1 X 15 min in PBS.
Cells are fixed again with 4% paraformaldehyde in PBS for 2 min at RT.
Wash 3 X 5min in PBS.
Dehydrate the slides in ethanol series: 70% ethanol for 2 min, 90% ethanol for 2 min, 100% ethanol for 2 min. Air-dry the slides.
Prepare hybridization solution (100 ul):

Formamide	75 ul
ddH20	16 ul
25 ug/ml PNA probe	1 ul
10% blocking buffer	7 ul (blocking buffer is the same as in Q-FISH & different from IF).
1M Tris pH 7.2	1 ul

Apply 50 ul per slide, and cover with coverslips.

Denature exactly for 3 min at 80oC (sometimes the hybridization works without heating, which helps morphology, I would try with and without).
Incubate for 2 h at RT in dark and humid conditions (humidity chamber).
Wash slides 2 X 15 min at RT in 50% formamide + 10 mM Tris pH 7.2 + 0.1% BSA solution.
Wash slides 2 X 15 min at RT in 1 X TBS + 0.08% Tween.
Dehydrate the slides in ethanol series: 70% ethanol for 2 min, 90% ethanol for 2 min, 100% ethanol for 2 min.
Air-dry the slides.
Cover slides with mounting media with DAPI and seal.