Immunofluorescence Microscopy of *S. pombe* cells.

Day1:

Cell culture in YES (10ml), 30˚C. (25˚C for ts cell lines)

Day2:

If cells are overgrown (OD: more than 1.5 in YES, 1.0 in EMM), dilute cells in medium at OD=0.2 and culture cells for two generation times (4h in YES, 6h in EMM; if cells grow like wild-type). Optimal condition for microscopic analysis is an OD of 0.5~1.0 in YES, 0.4~0.8 in EMM.

Fixation

Add 1ml of formaldehyde (30%) to the cell culture (10ml)
Vortex well and wait for 45sec @ RT
Add 100µl of glutaraldehyde (25%)
Vortex well and incubate for 1h @ 30˚C (@25˚C for ts) in a shaker
Centrifuge, 2000rpm, 3min, remove supernatant completely
Suspend cells in 1ml of PEM and transfer the suspension to 1.5ml tube
Wash cells in 1ml of PEM for three times
    {Centrifuge (10krpm, 30sec), remove sup, add 1ml of PEM}
At this point you have cells in 1ml of PEMS

Cell wall digestion, permeablization

Add 5ul of 200 x Zymolyase, vortex briefly to mix
Incubate, 70min, @37˚C, invert tube occasionally
    Check cells if cell wall is digested under the microscope
Centrifuge, 8krpm, 10sec
Add 200µl of PEMST, suspend cells gently, wait for 2min
Wash cells in PEM for three times
    {centrifuge (12krpm, 60sec), remove sup, add 200µl of PEM}

Quenching

Centrifuge, 12krpm, 60sec, remove sup
Suspend cells in 200µl of PEM containing 1mg/ml Sodium borohydride
Incubate, 5min, RT, with the lid open
Repeat the quenching steps another two times
    Preparation of Sodium borohydride solution
    Stand by 1mg of Sodium borohydride in a 1.5ml tube (prepare three tubes)
    Add 1ml of PEM just before quenching
    Use 200µl to suspend cells for one quenching reaction

Wash cells in 200µl of PEM for three times
    {centrifuge (12krpm, 60sec), remove sup, add 200µl of PEM}

Blocking, antibody reaction

Centrifuge (12krpm, 60sec), remove sup, suspend cells in 200µl of PEMBALG

Centrifuge (12krpm, 60sec), remove sup, suspend cells in 100µl of PEMBALG
Incubate for 1h @RT on the nutator
Centrifuge (12krpm, 60sec), suspend cells in 100µl of PEMBALG with the 1st antibody
Example: Anti-Myc (9E10), 1/2000
Incubate o/n @RT on the nutator

Day3:

Wash cells in 100µl of PEMBALG for three times
{centrifuge (12krpm, 60sec), remove sup, add 100µl of PEMBALG}
Centrifuge (12krpm, 60sec), remove sup, suspend cells in 100µl with 2nd antibody
Example: 1/200
Cover the tube with aluminum foil (2nd antibody is light sensitive)
Incubate o/n, RT, on the nutator

Day4:

Wash cells in 100µl of PEMBALG for three times
{centrifuge (12krpm, 60sec), remove sup, add 100µl of PEMBALG}
Centrifuge (12krpm, 60sec), remove sup, suspend cells in 50µ of PBS+0.2µg/ml DAPI
Immediately, Centrifuge (12krpm, 60sec), remove sup
Suspend cells in 20µl of PBS+0.02% Azide, store cells at 4˚C
You are now ready to go to the microscope room