Immunofluorescence Microscopy of Yeast

A. Spheroplasting Cells:

- Cut over night culture back to ~0.2 (15 ml culture) and grow to 0.6-0.8 OD (10 OD's total)
- Spin cells down in 15 ml conical tube and resuspend pellet in 2 ml Softening buffer, incubate 10 min at room temp.
- Spin and aspirate supernatant
- Add 2 ml Spheroplast Medium, add 15 μl Zymolyase (10 mg/ml), incubate 40 min at 30°C

B. Fix Cells

- Add formaldehyde to 4% to spheroplasted cells (220 μl of 37% stock), incubate 60 min at 30°C
- Spin in clinical 4 min at 1000 rpm (just hard enough to pellet the spheroplasts), aspirate, resuspend pellet in 1 ml Buffer A, transfer to Eppi
- Spin 2 min at 4000 rpm in eppi-fuge, aspirate, resuspend pellet carefully in 0.5 ml Buffer A + 0.1% SDS (or 1% LDAO from Fluka), incubate 10 min at room temp.
- wash 2x with 1 ml Buffer A (spin 2 min at 4000 rpm)
- resuspend in 200-400 μl Buffer A (dependent on size of pellet)

C. Preparing Slides (use a humidity chamber)

- Clean slide with canned air, apply round 0.5-inch coverslip to slide (add 2 μl water between slide and coverslip)
- Add 15 μl poly-L-lysine (2 mg/ml) to coverslip, incubate 10 min at room temp.
- Wash 4x with water
- Mix cells well (but carefully) and add 20 μl to coverslip, incubate 15 min
- Aspirate cells and block for 15 min by adding 20 μl Buffer B

D. Antibody Incubation (use a humidity chamber)

- Aspirate blocking Buffer B
- Add 15 μl 1° antibody (1/1000 to 1/10,000) in Buffer B (spin in Eppi centrifuge, avoid aggergates!), incubate 60-90 min at room temp. or over night at 4°C
- Wash 5x with Buffer B
- Add 15 μl 2° antibody in Buffer B (1/1000 for most 2° antibodies, spin in Eppi centrifuge, avoid aggergates!), incubate 60 min in dark at room temp.
- Wash 5x with Buffer B

E. Mounting

- On new slide add one drop of Mounting Solution
- Aspirate last wash from coverslip
- Dip coverslip into a beaker of water (use tweezers), remove excess water by touching the side of the coverslip with a kimwipe (remove water from tweezers!)
- Place coverslip cell side down onto the drop of Mounting Solution
- Remove excess Mounting Solution
- Seal with nail polish

Spheroplasting Medium:	YNB + 2% glucose + amino acids 1 M sorbitol 40 mM $KHPO_4$ buffer, pH 6.5
Softening Buffer:	0.1 M Tris pH 9.4 10 mM DTT
Buffer A:	100 mM Tris, pH 8 1M sorbitol
Buffer B:	50 mM Tris pH 8 150 mM NaCl 1% nonfat dry milk 0.5 mg/ml BSA 0.1% Tween 20 if stored add 1 mM NaN_3 **incubate on shaker for 30 min, remove particles by centrifugation at ~15000g for 10 min**
Mounting Solution:	for Alexa Dyes use 90% glycerol, PBS (1 ml 10x PBS + 9 ml glycerol)

Notes on primary antibodies:

Crude anti-Snf7: 1/10,000
Anti-Vps24 must be affinity purified