Preparation of Yeast Spheroplasts

Yeast cells have a polysaccharide wall that must be removed for many types of procedures (e.g., osmotic lysis). Yeast cells without a wall are referred to as "spheroplasts."

1. Grow cells in liquid culture to mid-logarithmic stage (0.5-0.8 ODU/ml)
   *cells at stationary phase have a thicker cell wall that is more difficult to remove
2. Centrifuge the cells at 5000 x *g* for 5 min at room temperature (RT)
3. Resuspend the cell pellet in sterile water at a concentration of 10 ODU/ml
4. Centrifuge at 5000 x *g* for 5 min at RT
5. Resuspend the cell pellet in "softening medium" at a concentration of 10 ODU/ml

   softening medium:
   100 mM Hepes-KOH, pH 9.4 (1 M stock, RT)
   10 mM dithiothreitol (DTT) (1 M stock, -20˚C)
   *always prepare fresh softening medium
   *Hepes-KOH may be substituted with another buffer (e.g., Tris pH 9.4 or Pipes-KOH pH 9.4)
6. Incubate for 15 min at RT
7. Centrifuge at 5000 x *g* for 5 min at RT
8. Resuspend cell pellet in "spheroplasting medium" at a concentration of 5 ODU/ml

   spheroplasting medium:

1X YNB	(10X stock, RT)
2% glucose	(50% stock, RT)
1X amino acids	(100X stock, RT)
50 mM Hepes-KOH, pH 7.2	(1 M stock, RT)
1 M sorbitol	(2 M stock, RT)

   *spheroplasting medium can be stored at RT indefinitely
9. Add zymolyase 100T to a final concentration of 2 µl/ODU
   *stock = 10 mg/ml in 1X PBS, 1 M sorbitol (store in aliquots at -80˚C)
10. Incubate for 60 min at 30˚C (or an otherwise appropriate temperature; for instance, if the strain has a temperature-sensitive growth defect, incubate at the permissive temperature)
    *mix solution briefly after 30 min of incubation
11. Centrifuge at 5000 x *g* for 5 min at RT
12. Resuspend spheroplasts in spheroplasting medium at concentration of 5 ODU/ml to remove zymolyase
13. Centrifuge at 5000 x *g* for 5 min at RT
14. You now have spheroplasts.