UGPase Activity Assay

Extract Preparation:

1. Grow 50 ml culture
2. Centrifuge at 3000 RPM for 5 min
3. Freeze at -80°C
4. Thaw/ Resuspend sample in 5 ml XWA/(50.0μl of 5M NaCl) +1:300PICs/1:300PhICs (16.5μl each in 5ml)
5. Add 3 ml (4g) glass beads
6. Vortex 1 min/Ice 1 min. Repeat 2 times (when you move to Oakridge tube add 1ml XWA to beads once empty, vortex and add it to tube)
7. Centrifuge at 12,000g for 20 min (setting 3)
8. Filter through 0.2μm filter
9. Load onto MonoQ column
10. Assay fractions from column

Assay:

1. Make master mix containing everything but extract and pyrophosphate

Per reaction (X40):

711 μl 10X reaction buffer

 0.5 M Tris pH 8.0

 100 mM DTT

 100 mM MgCl2

71 μl NADP (20 mM)

0.71 μl G-1,6 P2 (100 mM)

71 μl UDP-Glucose (200mM)

21.32 μl Phosphoglucomutase (0.4 U/μl) Roche 108374

7.12 μl G-6-P Dehydrogenase (1U/μl)

5.52 ml H2O

1. Aliquot 160 μl into each reaction tube (always include control w/o extract)
2. Add 20 μl of fraction from section1
3. Start reaction with 20 μl Na-pyrophosphate (100mM) tetrabasic Decahydrate sigma S-9515–Start reactions at 20 sec intervals to allow time for reading
4. Incubate reactions at 25°C for 10 min
5. Read abs at 340 nm/ subtract negative control