Purification of peroxisomes in a self-generated Percoll gradient

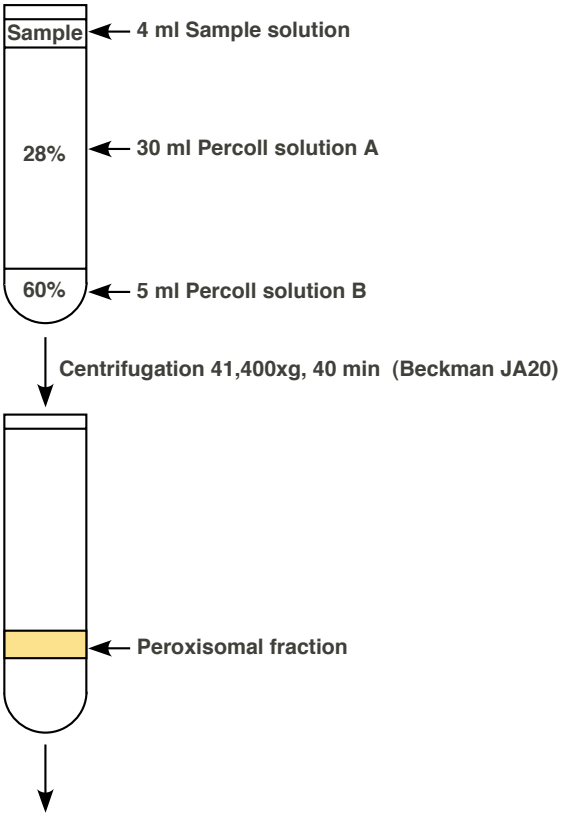
Homogenization buffer
20 mM Na pyrophosphate-HCl (pH7.5)
1 mM EDTA
300 mM Mannitol

Percoll solution A
28%(v/v) Percoll
10 mM HEPES-KOH (pH7.2)
1 mM EDTA
300 mM Mannitol

Percoll solution B
60%(v/v) Percoll
10 mM HEPES-KOH (pH7.2)
1 mM EDTA
300 mM Mannitol

Sample buffer
10 mM HEPES-KOH (pH7.2)
1 mM EDTA
300 mM Mannitol

25 g Arabidopsis cotyledons
↓ Homogenization with 100 ml of homogenization buffer
↓ Percolation by four layers of cheesecloth
Filtrate
↓ Centrifugation 1,500xg, 10 min (Beckman JA14)
Supernatant
↓ Centrifugation 10,000xg, 20 min (Beckman JA14)
Pellet
↓ Resuspend in 4 ml of sample buffer
Layer 4 ml of sample solution onto top of the Percoll solutions
Percoll (GE Healthcare) density gradient



Fractionation
SDS-PAGE
Identification of peroxisomal fractions by immunoblot analysis