Isolation of Oil Bodies

1 Reagents

1.1 Grinding medium 100 ml

EDTA	1	mM	⇒	500	mM	EDTA pH 8	0.2	ml
KCl	10	mM	⇒	1.0	M	KCl	1.0	ml
$MgCl_2$	1	mM	⇒	0.1	M	$MgCl_2$	0.1	ml
DTT	2	mM	⇒	1.0	M	DTT	0.2	ml
Tricine	0.15	M	⇒				2.67	g
Sucrose	0.6	M	⇒				20.54	g
KOH	pH 7.5							

1.2 Resuspension medium 100 ml

Grinding medium contains

Sucrose	0.6	M	⇒	20.54	g
NaCl	2.0	M	⇒	7.09	g
KOH	pH 7.5				

1.3 Floatation medium A 100 ml

Grinding medium contains

Sucrose	0.25	M	⇒	8.56	g
NaCl	2.0	M	⇒	7.09	g
KOH	pH 7.5				

1.4 Floatation medium B 100ml

Grinding medium contains

Sucrose	0.4	M	⇒	13.70	g
KOH	pH 7.5				

2 Preparation

Soak seeds in water for 1-24 hrs

Put all mediums on ice

Turn on the BECHMAN Ultracentrifuge

Prepare forcepts, vortex, mess pipet and pipetman

3 Protocoal

- Homogenize seeds at 277 K in Grinding medium 2.5 g/10 ml, aliquot 0.1 ml as F_1
- Filtration by nylon filter
- Put 6 ml of homogenate into a centrifuge tubes, aliquot 0.1 ml as F_2
- Gently lay 6 ml of Floatation medium B on top of the filtrated solution
- Spin tubes at 9,000 rpm for 30 min.
- Take supernatant and aliquot 0.1 ml as F_3, then resuspend it in 6 ml of Resuspension medium
- Gently lay 6 ml of Floating medium A on top of the solution, do not mix!
- Spin tubes at 9,000 rpm for 30 min
- for(int i =0; i++; i<2) {
    - Take supernatant and aliquot 0.1 ml as F_{i+4}, then resuspend it in 6 ml of Grinding medium
    - Gently lay 6 ml of Floatation medium B, do not mix!
    - Spin tubes at 9,000 rpm, 30 min

  }

Collect supernatant

Resuspend in Grinding medium to a concentration of 100 mg lipid/ ml

4 Memo

KCl = 74.557 g/mol

$MgCl_2 \cdot 6H_2O$ = 203.3 g/mol

EDTA· $2H_2O$ = 372.24 g/mol

Sucrose = 342.3 g/mol

Tricine = 179.2 g/mol

N-tris[Hydroxymethyl]methylglycine;N-[2-hydroxy1, 1-bis(hydroxymethyl)ethyl]glycine

DTT;DL-dithiothreitol

$F = ma = \frac{mv^2}{r}$

$x\ rpm = \frac{60v}{2\pi r}$

$G = 1.119 \times 10^{-3} r x^2$

A radious of roter SW41T1 is 110.2 mm

Operation of BECHMAN Ultra Centrifuge

- Turn on
- Clean up the water inside hatch and surface of the rotor
- Put rotor slowly and listen for a click
- Close the cover gently
- Set time, temp. and speed
- Turn on vacuume
- Wait for vacuum to reach > 2.0, and temperature to reach the desired setting (Ultracentrifuge will not start of vacuum or temp. is not at the desired level). Press start
- When the rotation stops, push the vacuum button to release the pressure
- Open the hatch
- Don’t forget to write down the record

Warburg-Christain Assay

Depends on Y and W amount

$A_{280} = E^{1\%}_{1cm} c \cdot l$

case l=1 cm

if $\frac{A_{280}}{A_{260}} > 1.5 \Rightarrow$ pritein /mg/ml=1 $0.5A_{280}$

else $\Rightarrow$ protein /mg/ml=1.45A_{280}-0.74A_{260}