Optiprep DG UC

homogenisation buffer (HM)
250 mM sucrose (21,4 g / 250 mL (MW:342,3 g/mol))
1 mM EDTA (500 uL 0,5 M EDTA / 250 mL)
10 mM Tris-HCL (0,394 g /250 mL (MW: 157,6 g/mol))
mix, pH: 7,4, dilute to 250 mL with MQ water, filter sterile in hood. stable at 4 °C for 3 months.

Working buffer (WB)
250 mM sucrose (21,4 g / 250 mL (MW:342,3 g/mol))
6 mM EDTA (3 mL 0,5 M EDTA / 250 mL)
60 mM Tris-HCL (2,36 g /250 mL (MW: 157,6 g/mol))
mix, pH: 7,4, dilute to 250 mL with MQ water, filter sterile in hood. stable at 4 °C for 3 months.

Working solution (WS):
1x WB + 5x Optiprep (Axis Shield, 60 % stock) >> this way you get a 50 % solution.
To run four tubes you need 4 x 1 mL, let's make 5 of each!
5%: 0,5 mL WS + 4,5 mL HM
10%: 1 mL WS + 4 mL HM
20%: 2 mL WS + 3 mL HM
40%: 4 mL WS + 1 mL HM
total 7,5 mL WS needed, let's make 8: 1,33 mL WB + 6,67 mL Optiprep stock (60%)

bottom to top layer into a 4,5 mL polyallomer MLS-50 tube:
1 mL 40%
1 mL 20%
1 mL 10%
1 mL 5%
max 0,5 mL sample on top (isolated, resuspended EXOs)
MLS-50 rotor, 100.000 g, 4°C, min 16 h!!!!!!
accel: 8
decel: COAST!!!!! (takes almost 45 minutes to stop!)

Next day:
prepare 9 pieces of EPP tubes (1,5 mL) for each gradient Tube (total 4 x9)
label them FR1-9 from top to bottom
measure each tube empty with the scale we use to balance the UC
write down all the weights of the tubes in a table
After stopping the UC collect 9 x 0,5 mL fractions from each tube top to bottom
measure their weight
calculate the density, the curve must be linear!!!!

Fraction	1	2	3	4	5	6	7	8	9
empty weight (g)									
weight with 0,5 mL sample (g)									
weight of sample (total – empty tube)									
denstity (sample weight / 0,5 mL)									

Dilute all fractions in one MLA-55 tube each (up to 8,6 mL)
spin again 100,000 g; 4°C; 3 hours, acc:8, dec:8!
collect your pellets