## Supplementary File 1

# ROTOR CALCULATIONS

$$\mathrm{RPM}=\sqrt{\frac{\mathrm{RCF}}{1.18r}}\times 1000 \qquad \text{(Eq.1)}$$

$$k=\frac{\left(2.533\times 10^{11}\right)\ln(r_{\mathrm{max}}/r_{\mathrm{min}})}{(\text{maximum RPM})^2} \qquad \text{(Eq.2)}$$

$$k_{\mathrm{adj}}=k\left(\frac{\text{maximum RPM}}{\text{actual RPM}}\right)^2 \qquad \text{(Eq.3)}$$

$$\frac{t_{\mathrm{A}}}{t_{\mathrm{B}}}=\frac{k_{\mathrm{A}}}{k_{\mathrm{B}}} \qquad \text{(Eq.4)}$$

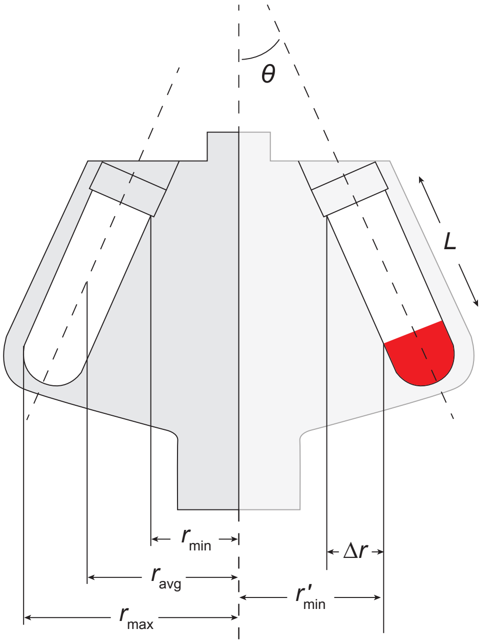
Equation 1 is used to calulate the revolutions per minute (RPM) from the applied g-force of the rotor (relative centrifugal force, RCF) where $r$ is the is the average radius of the particle to the axis of rotation. Equation 2 is used to calculate the $k$ factor (clearance factor) of the rotor where $r_{\mathrm{max}}$ and $r_{\mathrm{min}}$ are the maximum and minimum radius of the particle to the axis of rotation. Equation 3 is used to calculate the adjusted $k$ factor when the rotor is used at sub-maximal velocities. Equation 4 is the relation used to convert a centrifugal run for rotor A with centrifugation time $t_{\mathrm{A}}$ and clearance factor $k_{\mathrm{A}}$ to a second rotor B.

## Effective *k* factor for runs with tubes filled to less than maximum capacity

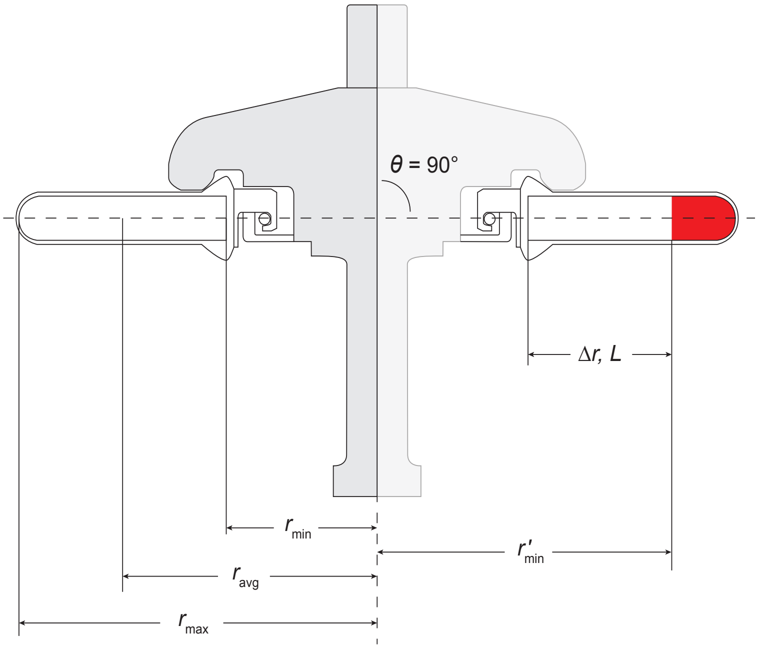
If centrifugation tubes are not filled to their nominal capacity the shorter path length that the average particle will have to travel before sedimentation will lower the effective $k$ factor, $k_{\mathrm{eff}}$ and thus lower the required run times. Specifically, filling tubes to less than capacity will increase the effective minimum radius to a new value $r'_{\mathrm{min}}$ that can be calculated as

$$r'_{\mathrm{min}}=r_{\mathrm{min}}+\Delta r \qquad \text{(Eq.5)}$$

where $\Delta r$ is the increase in minimum radius. The value of $\Delta r$ will depend on the tube angle to the axis of rotation $\theta$, and to $L$, the distance from the top of the tube to the actual fill level (in millimeters).



**Fixed angle rotor**



**Swinging bucket rotor**

For **fixed angle rotors**, $\Delta r$ will be equal to $L \times \cos(\theta)$ and thus the effective minimum radius can be calculated as

$$r'_{\mathrm{min}} = r_{\mathrm{min}} + L\cos\theta \qquad \text{(Eq.6)}$$

For **swinging bucket rotors**, the angle of the tube to the axis of rotation, $\theta$, is 90° so $\Delta r$ will be equal to the fill distance $L$, and the effective minimum radius becomes

$$r'_{\mathrm{min}} = r_{\mathrm{min}} + L \qquad \text{(Eq.7)}$$

For **near-vertical tube rotors**, the calculation of $r'_{\mathrm{min}}$ is the same as for the fixed angle rotors (Eq.6). The comparatively small angles to the axis of rotation for the near-vertical tube rotors results in small path lengths and therefore reduced run times compared to fixed angle rotors.

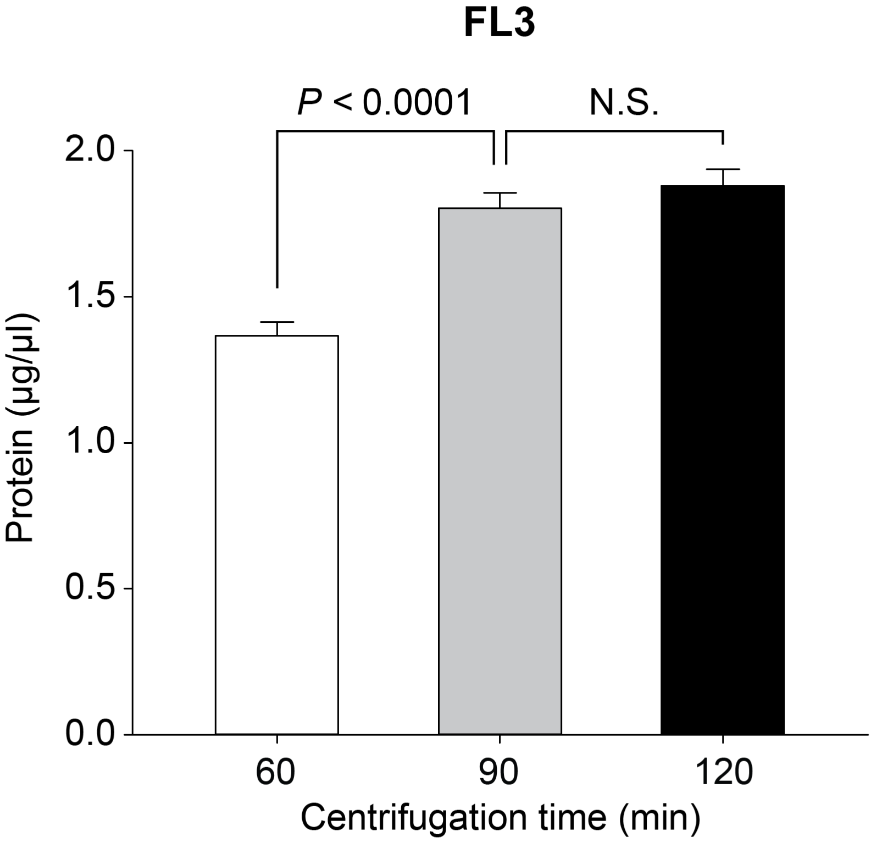
For **vertical tube rotors**, particles travel across the horizontal diameter of the tube rather than the length of the tube resulting in very short path lengths. Therefore, the path length that particles have to travel for sedimentation will be unaffected by fill level of the tube and so

$$r'_{\mathrm{min}} = r_{\mathrm{min}} \qquad \text{(Eq.8)}$$

The effective clearing factor $k_{\mathrm{eff}}$ of the rotor, with adjustments for the actual fill level of the centrifugation tube as well as sub-maximal speeds, can be then be calculated as

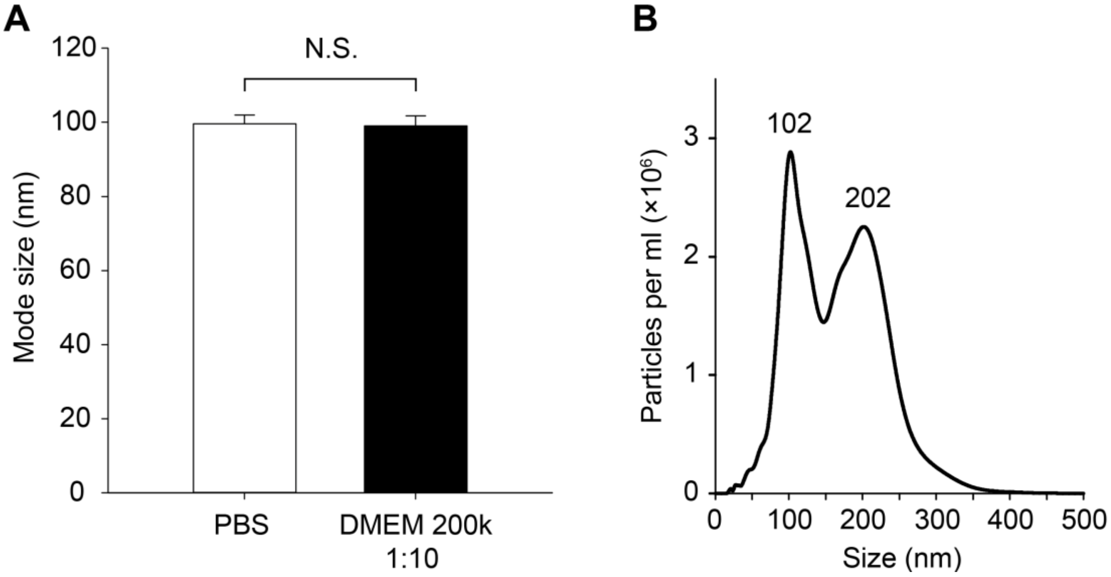
$$k_{\mathrm{eff}} = \frac{\left(2.533\times 10^{11}\right)\ln(r_{\mathrm{max}} / r'_{\mathrm{min}})}{\left(\text{actual RPM}\right)^2} \qquad \text{(Eq.9)}$$

# Supplementary Figures S1-S5



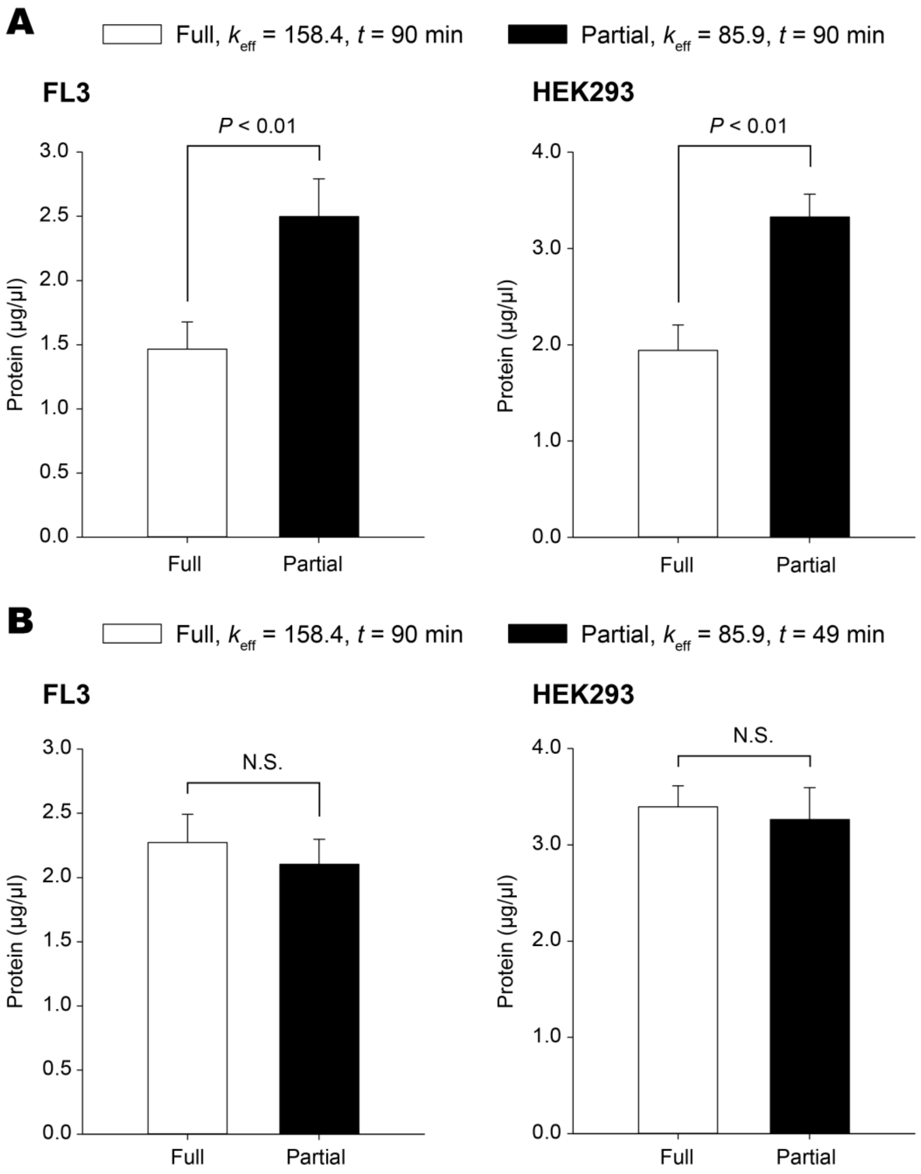
**Supplementary Figure S1**.

Quantification of protein in sedimentation pellets generated by 100k ultracentrifugation steps for 60, 90 or 120 min of pre-cleared FL3 CCM. Data is expressed as mean ± s.e.m. (n = 9 per group), *P*-value for overall difference between groups was determined by repeated measures ANOVA and *P*-values between pairs of groups were determined by Student's paired t-tests with correction for multiple comparisons by the Holm-Bonferroni procedure. N.S., not significant.



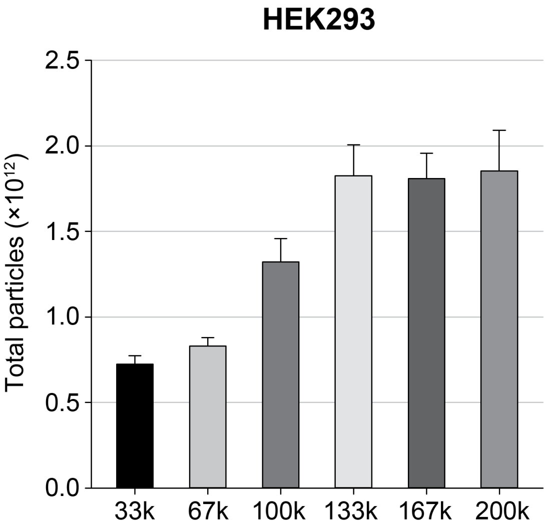
**Supplementary Figure S2**.

NTA measurements on control beads of defined size (A) The mode size of 100 nm of fluorescent polystyrene beads was measured in suspensions of either PBS or 200k-depleted DMEM without phenol red diluted 10-fold in PBS. Data is expressed as mean ± SD (n = 8 per group), *P*-value for difference between groups was determined by Student’s t-test. N.S., not significant. (B) The averaged size profile of particles in a PBS suspension of mixed 100 and 200 nm control beads (n = 3).



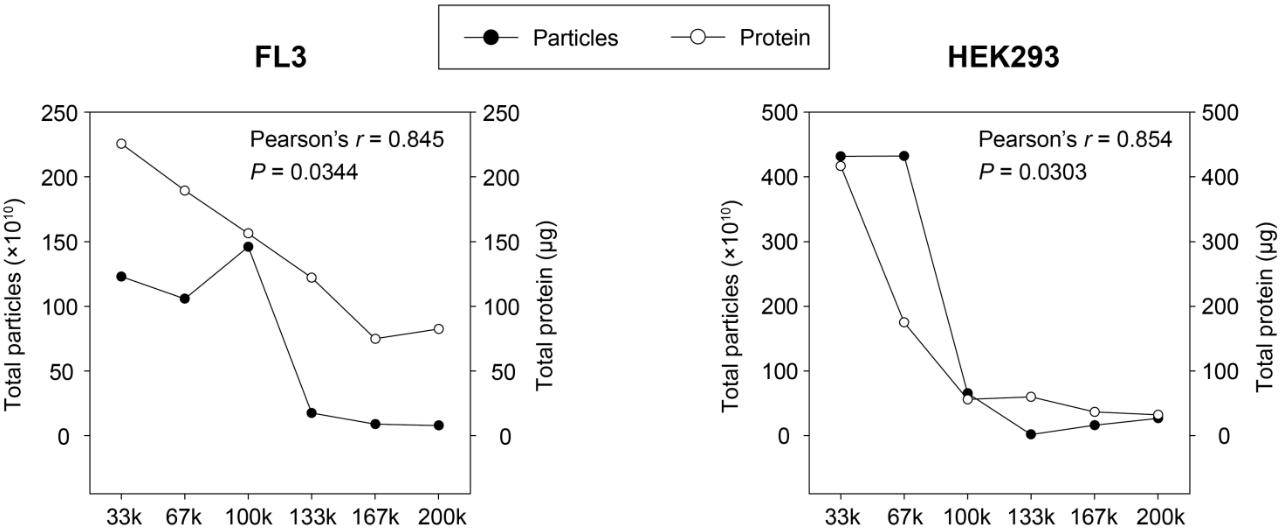
**Supplementary Figure S3**.

Quantification of protein in sedimentation pellets generated by 100k ultracentrifugation in a fixed-angle rotor using full tubes or partially filled tubes. Tubes were filled, either to nominal capacity (Full) or with a fill level to 20 mm from the top (Partial), with pre-cleared FL3 and HEK293 CCM. The effective *k* factors were calculated (Equations 6 and 9, Supplementary File 1). (A) Both the full and partial tubes were spun with a run time of 90 min Equal total volumes of CCM were processed for all samples. The protein content of the pelleted material was determined by Bradford micro assays. (B) The full tubes were spun with a run time of 90 min and the run time for the partially filled tubes for 49 min as calculated to obtain equivalent sedimentation (Equation 4, Supplementary File 1). Equal total volumes of CCM were processed for all samples. The protein content of the pelleted material was determined by Bradford micro assays. Data is expressed as mean ± SD. (n = 3 per group), *P*-value for difference between groups was determined by Student’s t-test. N.S., not significant.



**Supplementary Figure S4**.

NTA measurement of total number of particles in pellets resuspended in PBS after parallel ultracentrifugation of pre-cleared HEK293 CCM at speeds from 33k to 200k. Data is expressed as mean ± s.e.m (n = 5 per group).



**Supplementary Figure S5**.

Correlation between total number of pelleted particles and total protein in pellet after serial ultracentrifugation of FL3 (*left*) and HEK293 (*right*) pre-cleared CCM at speeds of 33k-200k. The linear correlation between particle number and protein was measured by Pearson's *r* (product-moment correlation coefficient). *P*-values for assessment of significance of correlation were determined by Student's t-test.