Centrifugation

Effortlessly transfer centrifugation protocols: simplifying your workflow

With the wide range of centrifugation rotors available on the market offering extensive speed and volume capabilities, it is crucial to understand some key centrifugation parameters to achieve consistent and replicable results. While some researchers may prefer to adhere to the platforms recommended in publications, there are instances where it becomes necessary to select a rotor with different specifications because of instrument availability or other factors. Therefore, understanding the process of calculating centrifugation parameters can help users easily transfer their existing protocol to a new platform that uses one of the many rotor options available on the market.

Understanding sedimentation path length and k-factor: key parameters for protocol transfer

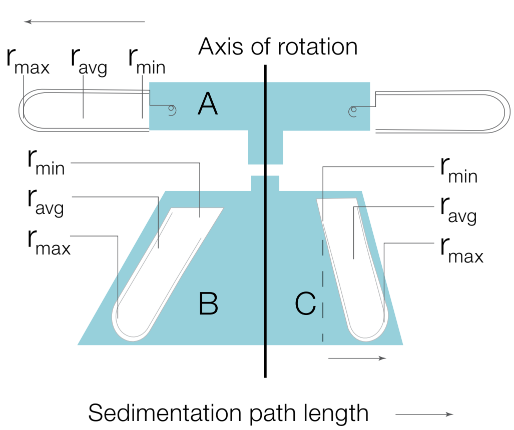

Figure 1. Comparison of r values of rotors. (A) Swinging-bucket, **(B)** fixed-angle, and **(C)** near-vertical rotors.

The sedimentation rates of particles can be calculated by determining the difference between the maximum radius (r_{max}) and the minimum radius (r_{min}) they reach during sedimentation. This difference is influenced by the rotor geometry and the size of the centrifuge tube (Figure 1).

The k-factor of a rotor, also known as pelleting efficiency, considers the sedimentation path length and its maximum RPM (Equation 1). The k-factor can be used to provide an estimated pelleting time (t) of known sedimentation coefficient (s_{20}) at the maximum speed of the rotor (Equation 2) [1,2].

Equation 1

$$k = 2.533 \times 10^5 \times \frac{\ln\left(\frac{r_{max}}{r_{min}}\right)}{\left(\frac{RPM}{1,000}\right)^2}$$

r_{max} = maximum radius (cm)

r_{min} = minimum radius (cm)

Equation 2

$$t = \frac{k}{s_{20}}$$

t = pelleting time (hours)

s_{20} = sedimentation coefficient in Svedberg units, determined in water at 20°C

k = k-factor at the maximum speed of the rotor

This information is useful for planning and optimizing centrifugation protocols, especially when working with different types of samples and rotors. By comparing the k-factors of two rotors and assuming use of the same sample, the run time for a particular separation can be estimated using Equation 3. These calculations utilize data from prior experiments where the k-factor (k_1) and run time (t_1) of one rotor are known [1,2].

Equation 3

$$t_2 = \frac{k_2 \times t_1}{k_1}$$

It is important to note that the k-factor does not account for acceleration and deceleration rates, and total run time may vary for different rotor materials. Furthermore, an adjusted k-factor must be calculated for speeds other than the maximum speed.

Transferring separation protocols

The process of transferring centrifugation protocols to different rotors, bottles, or tubes may initially appear challenging, but with the appropriate approach, it can be uncomplicated. By adhering to a few essential steps, the transfer of the protocol becomes a seamless activity.

Here we highlight the process of calculating centrifugation parameters to transfer a protocol for exosome isolation from conditioned medium that has been performed with the Beckman Coulter™ Type 70 Ti rotor at 100,000 x *g* for 70 minutes [3,4]. We show how to simply calculate new centrifugation parameters with alternative Thermo Scientific™ T-865, T-890, T-1250, and Fiberlite™ F37L-8x100 rotors.

Calculate RPM based on the average RCF

The first step is finding the average speed in RPM based on the protocol relative centrifugal force (RCF, also known as *g*-force). This can be achieved by converting RCF to RPM (Equation 4) using the protocol RCF and the average radius (r_{avg}) of the rotor. Table 1 highlights the average speed (RPM) at 100,000 x *g* for different rotors.

Equation 4

$$RPM = \sqrt{\frac{RCF}{1.118 \times 10^{-5} \times r}}$$

Calculate the adjusted k-factors

The critical step in protocol transfer is finding the adjusted k-factor. The k-factor that is listed under each rotor specification is determined at the maximum speed of the rotor. For instance, the k-factor of the Type 70 Ti rotor is 44 when operated at 70,000 RPM (504,000 x *g*, Table 1). For exosome isolation from cell culture medium, an RCF of 100,000 x *g* should be maintained. At higher RCF, other unwanted molecules will precipitate. Therefore, to compare rotor efficiencies and determine the centrifugation run time using Equation 3, the adjusted k-factor at 100,000 x *g* must be calculated using Equation 1. In this case, Equation 1 uses the average speed at 100,000 x *g* (e.g., 36,914 RPM for the Type 70 Ti rotor).

Table 1 lists adjusted k-factors for Type 70 Ti, T-865, T-890, and T-1250 rotors at 100,000 x *g*. Because of similar sedimentation path lengths for Type 70 Ti, T-865, and T-1250 rotors, the adjusted k-factors are similar. On the other hand, the T-890 rotor has a lower k-factor at 100,000 x *g* because of its shorter path length.

Calculate the centrifugation time

The final step is calculating the run time of new rotors based on the adjusted k-factor by using Equation 3. In this scenario, k_1 stands for the adjusted k-factor of the Type 70 Ti rotor at 100,000 x *g*, and t_1 stands for the centrifugation time recommended in the protocol (70 minutes). As an example, centrifugation time for the T-865 rotor at 100,000 x *g* can be calculated as follows:

$$t_2 = \frac{k_2 \times t_1}{k_1} = \frac{157.9 \times 70 \text{ min}}{157} = 70.4 \text{ min} = \sim 70 \text{ min}$$

Centrifugation times for exosome isolation using other rotors have also been calculated and are provided in Table 2.

Summary of protocol transfer to different rotors

The T-865 rotor can be utilized to isolate exosome vesicles from conditioned medium within 70 minutes at 100,000 x *g*. Although the unadjusted k-factor of the T-1250 rotor is ~1.6 times higher than that of the Type 70 Ti rotor (68.7 vs. 44), the T-1250 rotor requires similar time for separation to obtain the same outcome.

Alternatively, opting for a higher-performance rotor like the T-890 rotor allows for the collection of exosome particles at an average of 100,000 x *g* within a shorter time frame of 56 minutes.

When dealing with an increased sample volume and considering a rotor with higher sample capacity on a newer platform, the Fiberlite F37L-8x100 rotor is a suitable choice. This rotor facilitates the harvesting of 800 mL of the sample at 100,000 x *g* within 96 minutes. While the run time is extended because of the higher adjusted k-factor, the higher sample capacity may help save protocol time when handling large volumes. Table 2 summarizes the centrifugation parameters calculated for 4 different rotors. These parameters should provide similar results as the Type 70 Ti rotor when harvesting exosomes from conditioned cell culture medium.

Table 1. Comparison of centrifugation parameters of different rotors.

Rotor	Type 70 Ti	T-865	T-890	T-1250	Fiberlite F37L-8x100
Rotor capacity	8 x 39 mL	8 x 36 mL	8 x 12.5 mL	12 x 36 mL	8 x 100 mL
Maximum speed (RPM)	70,000	65,000	90,000	50,000	37,000
Maximum radius (cm) (r_{max})	9.19	9.1	7.65	10.78	11.9
Minimum radius (cm) (r_{min})	3.95	3.84	3.42	5.47	4.8
Average radius (cm) (r_{avg})	6.57	6.47	5.54	8.13	8.35
Maximum RCF	504,000 x *g*	429,459 x *g*	692,149 x *g*	301,032 x *g*	182,460 x *g*
Path length (cm)	5.24	5.26	4.23	5.3	6.54
k-factor	44	51.7	25.1	68.7	168
Average speed (RPM) at 100,000 x *g**	36,914	37,198	40,236	33,103	32,700
Adjusted k-factor at 100,000 x *g*	157	157.9	126	156.8	215
Run time at 100,000 x *g*	70 minutes	70 minutes	56 minutes	70 minutes	96 minutes

* Speed calculated is based on r_{avg}, which is $(r_{max} + r_{min})/2$.

Table 2. Centrifugation parameters for the exosome isolation protocol.

Rotor	T-865	T-890	T-1250	Fiberlite F37L-8x100
Average RCF (x *g*)	100,000	100,000	100,000	100,000
Speed (RPM)	37,198	40,236	33,103	32,700
Centrifugation time	70 minutes	56 minutes	70 minutes	96 minutes

Conclusion

Although transferring centrifugation protocols to new rotors may appear challenging, it can be quite straightforward when approached correctly. The k-factor that correlates sedimentation path length and rotor geometry with speed is one of the key centrifugation parameters that should be used in protocol transfer. It is important to adjust this parameter based on the protocol RCF. If the k-factor listed in the rotor specifications is used directly in the calculation of centrifugation run time for the new platform, the original protocol conditions may not be reproduced correctly. However, by carefully performing a few simple calculations, users can easily transfer their existing centrifugation protocols to new platforms.

References

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