ALFA WASSERMANN

Separation Technologies

KII & PKII

Ultracentrifuge Rotor Assemblies

Rotor Specification Tables

KII Rotors

Rotor Type	Application	Max. Force	Capacity with Core	Dimensions
 K3	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	3.2 liters	Diameter: Max:130 mm / 5.2″ Min: 110 mm / 4.3″ Path Length: 11 mm / 0.45″
 K5	For separation using rate zonal techniques. Separation is based on sedimentation rates of material being separated. Sequential rate zonal and isopycnic banding centrifugation permits a two dimensional separation to be made on the basis of both particle size and continuous flow rotor.	At 40 500 rpm Rmax: 121 200 xg Rmin: 38 500 xg K factor 177	8.4 liters	Diameter: Max: 130 mm / 5.2″ Min: 42 mm / 1.6″ Path Length: 45 mm / 1.7″
 K6	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. Integral pre-clarifier allows the initial capture of heavy particles such as whole cells or cell debris, and eliminates the need for a pre-clarification step.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7 Pre-clarifier: Rmax: 53 900 xg Rmin: 49 000 xg	3.2 liters Pre-clarifier: 0.17 liters	Diameter: Max: 130 mm / 5.2″ Min: 110 mm / 4.3″ Path Length: 11 mm / 0.45″ Pre-clarifier Diameter: Max: 58 mm / 2.3″ Min: 23 mm / 0.94″
 K10	Separation of large volumes of solids by isopycnic banding techniques. Basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 38 500 xg K factor 140	8.0 liters	Diameter: Max: 130 mm / 5.2″ Min: 53 mm / 2.1″ Path Length: 39 mm / 1.5″
 K11	Separation of low molecular weight sub-cellular components. Very short sedimentation path permits the separation of extremely small particles	At 40 500 rpm Rmax: 121 200 xg Rmin: 121 100 xg K factor .012	0.38 liters	Diameter: Max: 130 mm / 5.2″ Min: 129.5 mm / 5.2″ Path Length: 0.5 mm / 0.02″

Rotor Assemblies for Rate Zonal and Isopycnic Banding Techniques

PKII Rotors

Rotor Type	Application	Max. Force	Capacity with Core	Dimensions
 PK3	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	1.6 liters	Diameter: Max:130 mm / 5.2” Min: 110 mm / 4.3” Path Length: 11 mm / 0.45”
 PK3S	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. The same separation profile is obtained as for the PK-3 but with reduced process volume allowing for experimental runs from as low as 5 liters.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7	0.8 liters 0.4 liters 0.2 liters	Diameter: Max:130 mm / 5.2” Min: 110 mm / 4.3” Path Length: 11 mm / 0.45”
 PK6	For separation using isopycnic banding techniques with viral particles, virus like particles, nano-spheres. Integral pre-clarifier allows the initial capture of heavy particles such as whole cells or cell debris, and eliminates the need for a pre-clarification step.	At 40 500 rpm Rmax: 121 200 xg Rmin: 100 000 xg K factor 29.7 Pre-clarifier: Rmax: 53 900 xg Rmin: 49 000 xg	1.6 liters Pre-clarifier: 0.17 liters	Diameter: Max: 130 mm / 5.2” Min: 110 mm / 4.3” Path Length: 11 mm / 0.45” Pre-clarifier Diameter: Max: 58 mm / 2.3” Min: 23 mm / 0.94”
 PK10	Separation of large volumes of solids by isopycnic banding techniques. Basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 38 500 xg K factor 140	4.0 liters	Diameter: Max: 130 mm / 5.2” Min: 53 mm / 2.1” Path Length: 39 mm / 1.5”
 PK11	Separation of low molecular weight sub-cellular components. Very short sedimentation path permits the separation of extremely small particles	At 40 500 rpm Rmax: 121 200 xg Rmin: 121 100 xg K factor .012	0.19 liters	Diameter: Max: 130 mm / 5.2” Min: 129.5 mm / 5.2” Path Length: 0.5 mm / 0.02”

Influenza Virus Vaccine | K3 Rotor

Capture rate: 95% Recovery: 70% Purification factor: x 50

Centrifuge System	KII
Rotor System	Titanium rotor with K3 Noryl Core. Total volume (with core) is 3200 ml.
Type of Separation	Continuous flow with banding.
Gradient	One step sucrose gradient: 1600 ml of 60% w/w Sucrose and 1600 ml buffer. Buffer: Buffered Saline. Quantity should be 2.4 liters / run.
Buffer	Buffer is used to fill the remaining volume of the rotor (before the gradient material) and is used to establish flow during acceleration to set speed. The dynamic flow rate is 15 l/h.
Starting Material	The viral and non-viral material in allantoic fluid. Volume is typically 150 to 200 liters / run. Flow rate at speed is 15 to 25 l/h.
Operation	Operating speed is 35 000 rpm and the maximum centrifugal force is 90 000 xg. After all the material has been processed through the rotor, the trapped material should be allowed to band for at least one hour.
Unloading	The rotor is unloaded statically in 100 ml fractions.
Clean-out	Experienced clean out ranges around 95 to 100%.
Comments	A pre-clarification step may be necessary this depends on the amount of particulate material in the process fluid.

Rabies Virus Vaccine | PK3 Rotor

Capture rate: 95% Recovery: 90% Purification factor: x 90

Centrifuge System	PKII
Rotor System	Titanium rotor with PK3 Noryl Core. Total volume (with core) is 1600 ml.
Type of Separation	Continuous flow with banding.
Gradient	One step sucrose gradient: 800 ml of 60% w/w Sucrose and 800 ml buffer. Buffer: Buffered Saline. Quantity should be 1.2 liters / run.
Buffer	Buffer is used to fill the remaining volume of the rotor (before the gradient material) and is used to establish flow during acceleration to set speed. The dynamic flow rate is 6 l/h.
Starting Material	Viral and non-viral material in allantoic fluid. The volume is typically 75 to 100 liters / run. The flow rate at speed is 7.5 to 12.5 l/h.
Operation	Operating speed is 35 000 rpm and the maximum centrifugal force is 90 000 xg. After all the material has been processed through the rotor, the trapped material should be allowed to band for at least one hour.
Unloading	The rotor is unloaded statically in 50 ml fractions.
Clean-out	Experienced clean out ranges around 95 to 100%.

Hepatitis B Vaccine | K5 Rotor

Capture rate: 100% Recovery: 80% Purification factor: x 10

Centrifuge System	KII
Rotor System	Titanium rotor with K5 Noryl Core. Total volume (with core) is 8400 ml.
Type of Separation	Batch separation.
Gradient	One step sucrose gradient: 4400 ml of 55% w/w Sucrose and 4400 ml product. Buffer: Product should be buffered to improve the stability of the product.
Buffer	Product stabilized in buffer is used to fill the remaining volume of the rotor (before the gradient material).
Starting Material	Viral and non-viral material in clarified cell culture fluid.
Operation	Operating speed is 30 000 rpm and the maximum centrifugal force is 66 500 xg. Run time is 8 hours at speed.
Unloading	The rotor is unloaded statically in 500 ml fractions.
Clean-out	Experienced clean out ranges around 95 to 100%.

Adenovirus Vector | PK3-400 Rotor

Capture rate: 95% Recovery: 70% Purification factor: x 20

Centrifuge System	PKII
Rotor System	Titanium rotor with PK3-400 Noryl Core. Total volume (with core) is 400 ml.
Type of Separation	Continuous flow with banding.
Gradient	One step Nycodenz gradient: 200 ml of 40% w/w Nycodenz and 200 ml buffer. Buffer: Buffered saline. Quantity should be 0.6 l / run.
Buffer	Buffer is used to fill the remaining volume of the rotor (before the gradient material) and is used to establish flow during acceleration to set speed. The dynamic flow rate is 10 l/h.
Starting Material	Viral and non –viral material in cell culture fluid. The volume is typically 20 liters. Flow rate at speed is 10 l/h.
Operation	Operating speed is 35 000 rpm and the maximum centrifugal force is 90 000 xg. After all the material has been processed through the rotor, the trapped material should be allowed to band for at least one hour.
Unloading	The rotor is unloaded statically in 15 ml fractions.
Clean-out	Experienced clean out ranges around 95 to 100%.

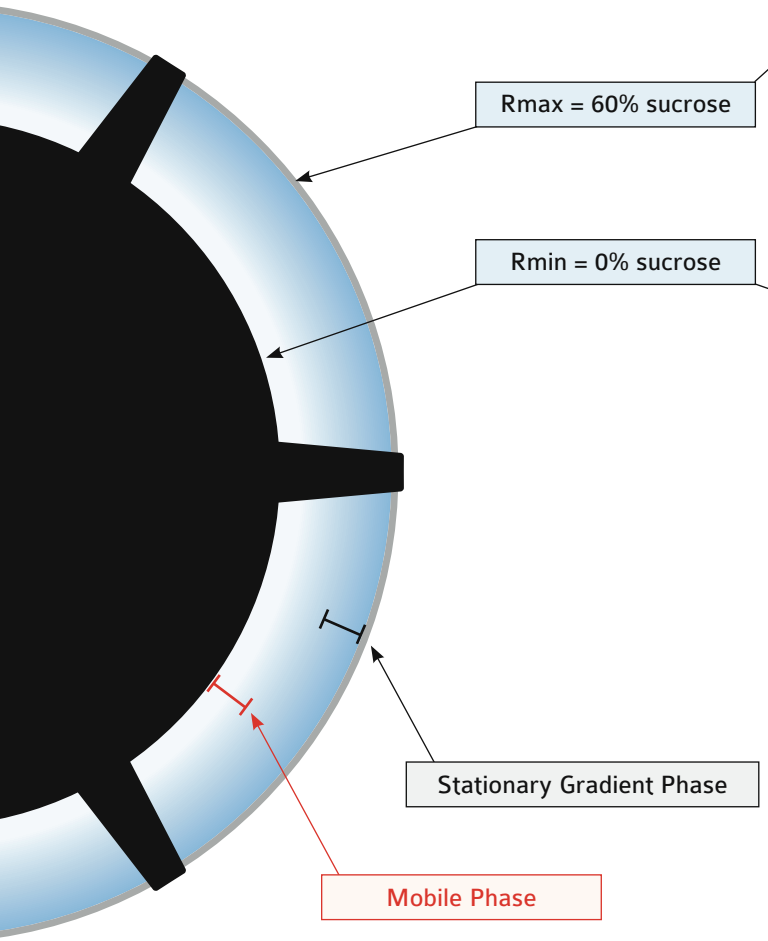
Zonal Density Gradient Reorientation

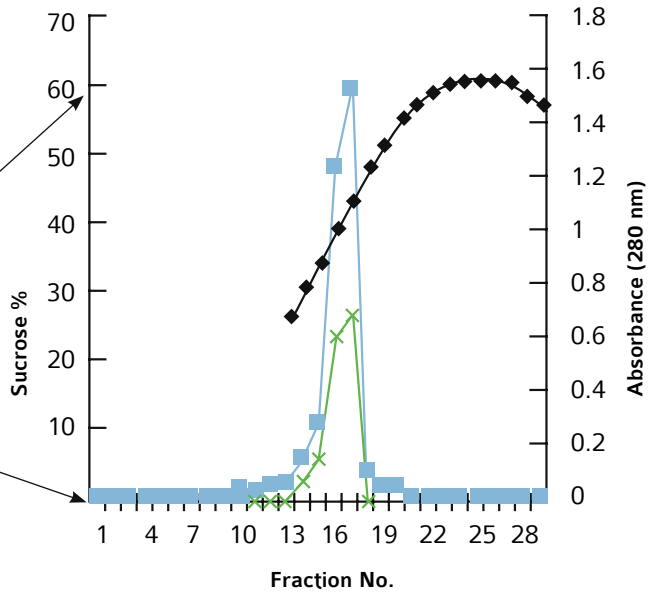
Linear Scale Up for Bioprocess Development

Ultracentrifugation density gradient particle purification can easily be scaled up from a laboratory protocol to manufacturing volumes using the 'K3' series of rotors. An experiment performed using a 100 ml rotor can be directly scaled to 3200 ml with only parameter change being the flow rate during continuous product feed.

Scale up from the laboratory volume (V_L) to the manufacturing volume (V_M) is easily determined.
E.g. PK3-400 running at a flow rate (F_M) of 2 l/h scale up to the K3 rotor with volume 3.2 l.

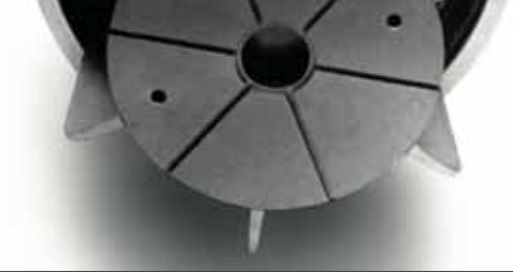
$$FL = \frac{V_M \times F_M}{V_L} = \frac{3.2 \times 2}{0.4} = 16 \text{ l/h}$$



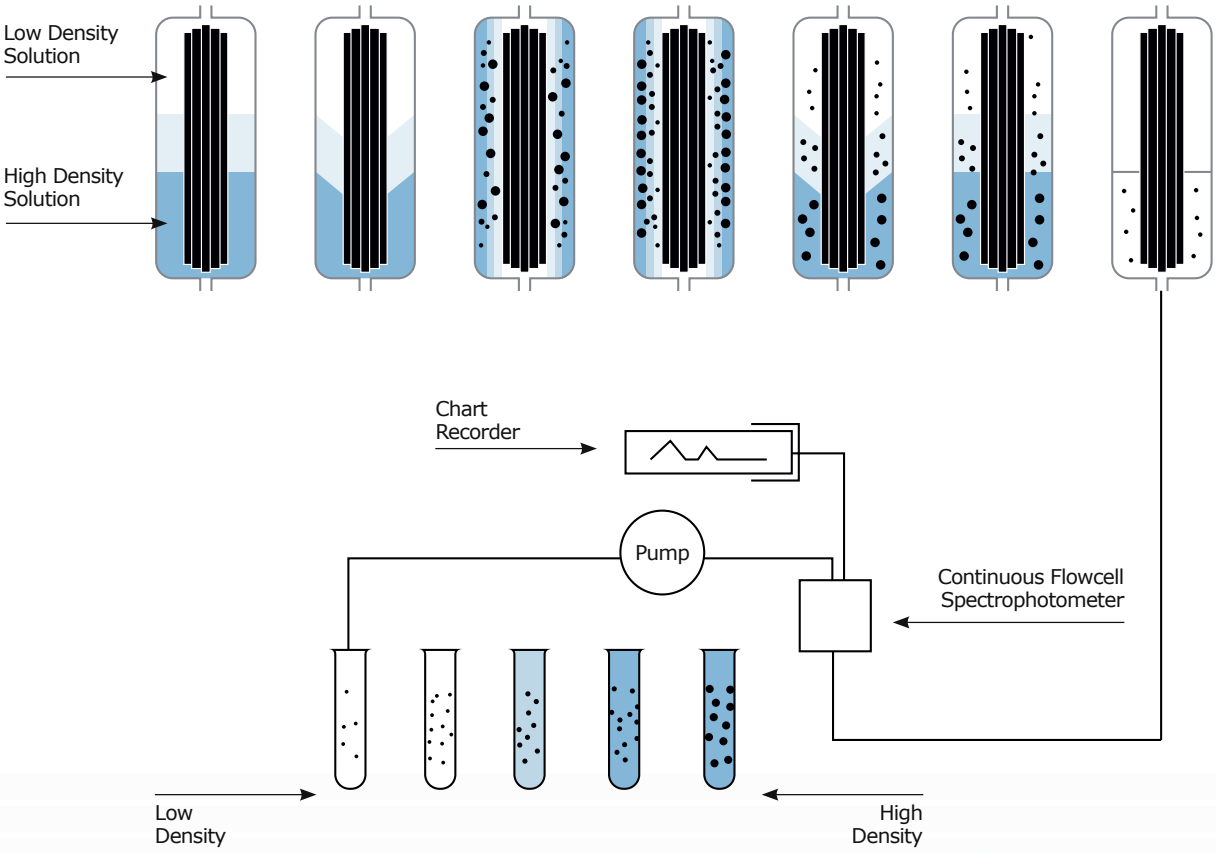


1600 ml Core

Results from a PK3 Rotor separation after fractionation showing the product peak in the density gradient of 0 to 60% w/w sucrose.



Stage	Process
1	The density gradient is loaded into the rotor while it is at rest.
2	As the rotor is gradually accelerated, the gradient reorients itself vertically along the outer wall of the rotor assembly.
3	Once at operating speed sample fluid is pumped into the rotor on a continuous flow basis.
4	The sample particles sediment radially into the gradient of increasing density. They eventually band (iso-pycnically) in cylindrical zones where the gradient density equals a particle's buoyant density.
5	At the end of the run, the rotor is decelerated to rest.
6	The gradient reorients itself to the original position without disturbing the particle bands.
7	The banded particles are now ready to be unloaded. Fractions are collected using air or water pressure and a pump to control flow.



Centrifugation Principles

K Factor and Sedimentation Time Calculation

The ultracentrifuge rotors run at an operating speed of 40 500 rpm while maintaining continuous flow-through capabilities. The relative centrifugal force (RCF) generated increases with radius from the center of rotation, giving a maximum RCF at the edge of the rotor of 121 200 xg. The pelleting efficiency of any rotor, at a given RPM, is represented by its K-factor, which can be calculated from the following equation:

$$\text{K-factor} = \frac{(2.53 \times 10^5)\ \ln\left(\frac{R_{max}}{R_{min}}\right)}{\left(\frac{RPM}{1000}\right)^2}$$

1

ln = natural log
Rmax = max. Radius
Rmin = min. Radius
RPM - rev. per minute

A lower K-factor represents a higher pelleting efficiency. The K-factor is extremely useful in estimating the pelleting time (T) for a given particle of known sedimentation coefficient (S):

$$T\ (h) = \frac{K}{S}$$

2

The short sedimentation path length and high centrifugal force in the rotors make them especially efficient. The K factors at maximum speed are tabulated in the rotor specification tables.

Sedimentation time calculations for the K3 rotor running at 40 500 rpm	
Influenza 700S	Time to sediment: 2 min 33s
Rabies 120S	Time to sediment: 14 min 54s
Adenovirus 200S	Time to sediment: 8 min 54s
Lentivirus 120S	Time to sediment: 14 min 54s
Vaccinia 1000S	Time to sediment: 1 min 47s

Calculation of Viral Capture Rate

The operational flow rate for removal of viral particles from cell culture harvest material can be calculated when the sedimentation coefficient of the particle of interest is known.

For the removal of Influenza virus (700S) from cell culture media on a continuous flow basis the K3 rotor is selected. Running the rotor at the maximum operating speed of 40 500 rpm the time required to pellet the virus in the rotor can be calculated using equation (2):

$$T\,(h) = \frac{K}{S} = \frac{29.74}{700} = 0.042\ h\ (2\ min\ 33s)$$

This means that for Influenza virus particles a minimum of 3 minutes and 33 seconds residence time is needed to allow sufficient time for the virus particles to sediment into the stationary gradient phase.

The K3 rotor assembly working volume is 3.2 liters, loaded to this will be a density gradient media e.g. Sucrose. The volume of gradient loaded will occupy 50% of the working volume leaving the remainder as the flow through volume (FT) so the flow rate to sediment the virus can be determined:

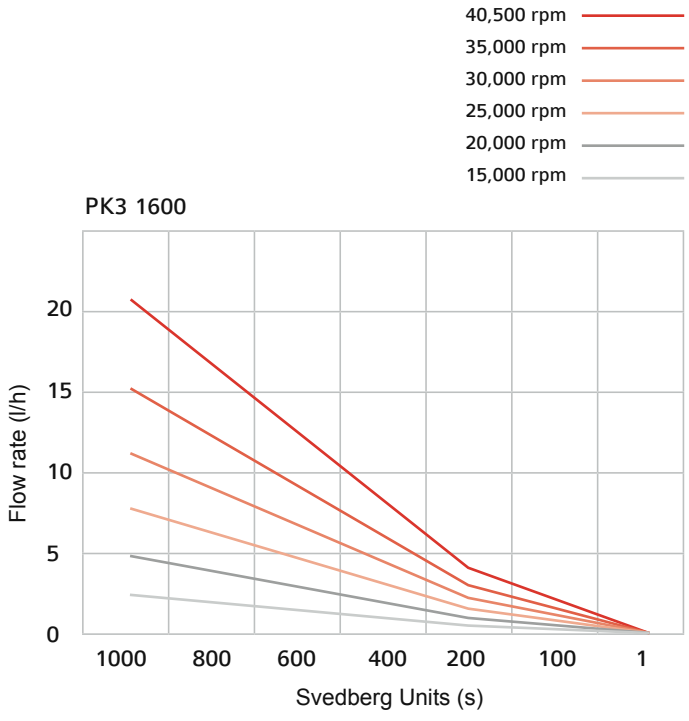
3

$$\text{Operational Flow Rate (l/h)} = \frac{FT}{T} = \frac{1.6\ l}{0.042\ h} = 38\ l/h$$

The theoretical predictions of virus removal in fact hold true in practice, the efficiency being reduced by the viscosity of the feed stream which will require a lower flow rate to capture virus. For example, at a rate of 10 l/h at least 6 logs of BVD can be removed from infected bovine serum. Other larger viruses like HTLV-III (650S) are easily removed from serum or purified on sucrose gradients. Influenza A virus (700S) is easily purified on a sucrose gradient from large volumes of allantoic fluid, a process that is the standard method for influenza vaccine production.

Separation flow is easy to determine

Influenza 700S
Adenovirus 200S
Vaccinia 1000S
Rabies 120S
Lentivirus 120S



Protocols using the KII Ultracentrifuge

Examples of virus isolation protocols :

Adenovirus
Hepatitis B
HBLV
Influenza
Rabies
NDV, Mumps
RSV, MULV, MOMLV, AKRMLV
Japanese Encephalitis
Polio
Vaccinia

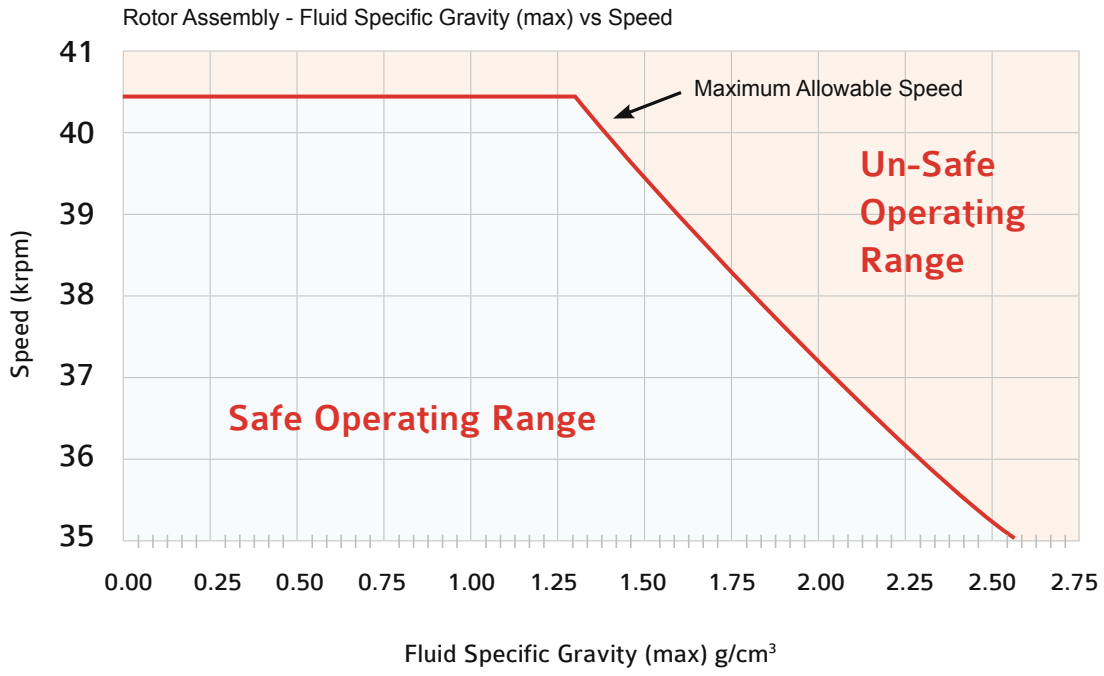
Speed vs Density Derating

Density gradient solutions with specific gravity ranging up to 1.6 g/cm³ can be safely used in the ultracentrifuge rotor to create gradients for separation protocols. When the specific gravity is greater than 1.3 g/cm³ it is necessary to reduce the maximum allowable running speed as outlined in the diagram below. For specific applications the following equation can be used to determine the maximum allowable run speed for a particular gradient material.

4

$$\text{Derated Speed} = \frac{\text{Maximum Rated Speed (rpm)} \times \sqrt{1.3}}{\text{max density at Rmax}}$$

Rotor maximum fluid specific gravity vs speed



Lifetime

All rotors may be operated at the maximum speed indefinitely with no need for periodic derating when they are maintained in a rotor maintenance contract. The customer must inspect the rotor routinely during operation for any signs of corrosion pits or cracks. Particularly attention should be paid to the areas around fluid passages and O-ring grooves, and the inside surfaces of the bowl and bowl caps. Any evidence of corrosion or cracking should be brought to the attention of Alfa Wassermann.

Alfa Wassermann rotors are warranted for 1000 hours at full rated speed or one year, whichever comes first, regardless of the number of runs, and provided that no corrosion is present. The only condition is that the customer adheres to the instructions for rotor use, maintenance and inspection contained in the manual, and maintains a daily log or record of rotor use.

Cleaning

Assembly of the rotors should be made on the rotor cart for ease of handling. Never let a rotor stand filled with fluids for extended periods of time when this is not necessary. The cleaning procedures must be followed carefully to prevent corrosion and prolong the life of the rotor. For normal cleaning the rotor should be rinsed in warm distilled water and dried thoroughly.

Sterilization

All rotor assemblies are compatible with a wide range of disinfecting and sanitizing agents. Any sterilization method, including steam sterilization is permitted for Titanium rotors and cores. When Noryl® cores are used then sterilization by steam is limited to 105°C. Other methods include exposure to ethylene oxide and ultraviolet radiation.

Noryl® cores must not be exposed to hydrocarbon solvents, for processes requiring the use of hydrocarbon solvents rotor cores made of either Titanium or PEEK should be used.

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