

KII & PKII ULTRACENTRIFUGE SYSTEMS

Solutions for Process Development
to Industrial Scale Manufacturing

Revolutionize Downstream Processing With Linear Scale Continuous
Flow Centrifugation



Continuous Flow Ultracentrifuges – Meeting all Your Demands from Application Development to Industrial Scale Production



PKII ULTRACENTRIFUGE



KII ULTRACENTRIFUGE

PKII Ultracentrifuge

PROCESS DEVELOPMENT AND SMALL SCALE PRODUCTION

Linear Scale Technology – Revolutionize your downstream process with linear scale-up

Process Development begins at a small scale and quickly moves to the manufacturing scale. To meet the needs of rapid and efficient scale-up, we have a new series of 'scale rotors' to match the volume you need in your process.

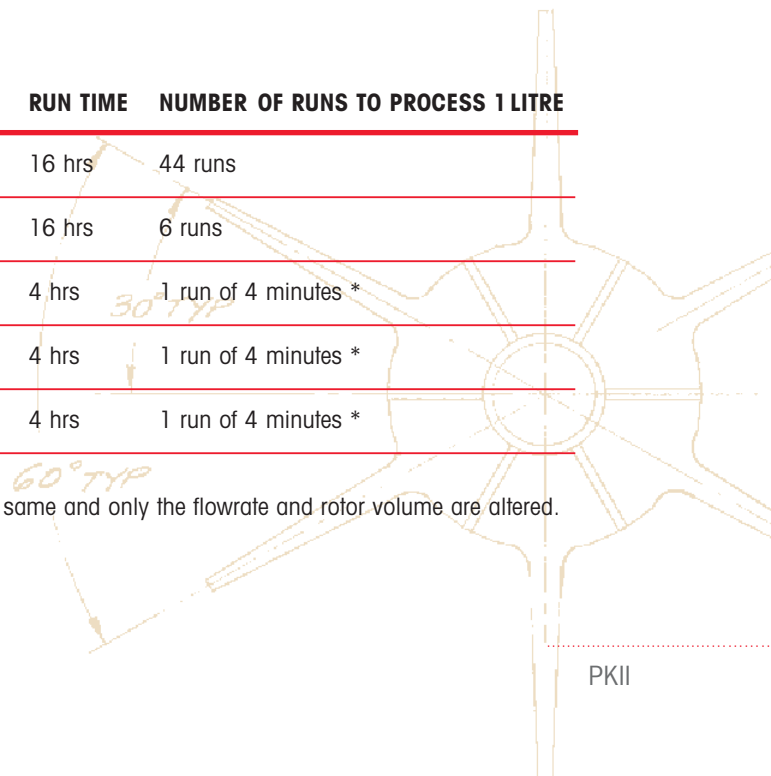
- > Rotor Volume 200 ml to 4000 ml
- > Linear Rotors 200 ml to 1600 ml
- > Process Volume 1L to 100 L
- > Speed up to 40,500 rpm (121,000 xg)
- > Multiple Rotor Formats

The PKII fits into the laboratory environment by having a low sample volume. In the same centrifuge, both experimental lots and production lots can be manufactured by increasing volume while retaining the purification profile using the unique scale core design. The scale-up is rapid because the resolution of product and impurity is identical at all operational volumes.

The PKII Can Process 1 Litre of Harvest Material in 4 Minutes

VOLUME TO PROCESS	ROTOR TYPE	ROTOR VOLUME	RUN TIME	NUMBER OF RUNS TO PROCESS 1 LITRE
23 ml	Swing Out	38 ml x 6 tubes	16 hrs	44 runs
160 ml	Batch Zonal Rotor	1600 ml	16 hrs	6 runs
1 to 20 L	PKII Lab Continuous Flow	200 ml	4 hrs	1 run of 4 minutes *
20 to 50 L	PKII Continuous Flow	400 ml	4 hrs	1 run of 4 minutes *
50 to 100 L	PKII Continuous Flow	1600 ml	4 hrs	1 run of 4 minutes *

* Rotors for the PKII are all linear scale therefore process run time is the same and only the flowrate and rotor volume are altered.



Commercial scale production can now be modeled at the laboratory scale enabling rapid bioprocess scale-up

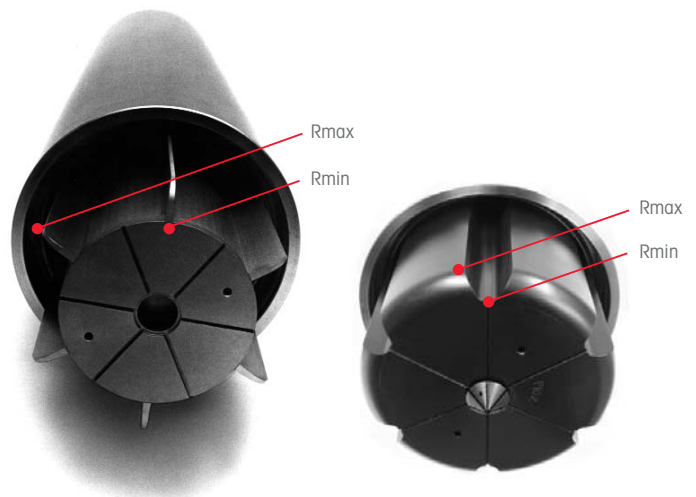
Linear Scale experiments significantly reduce process development time and cost

An experiment performed at the 200 ml rotor volume can be directly scaled-up to the 3200 ml rotor used at full scale production in the KII. The process volume for an experiment can be as little as 1 L starting material; full scale production can be 200 L per rotor run. Process time on scale-up remains the same as experimental lots, only the flow rate of operation is linearly increased. This means the following:

- > Direct comparison of all results
- > No loss of product resolution
- > No loss of yield with scale
- > Equal process times ensures stability of the product is not lost on scale-up

Separations in the centrifuge rotors are caused by the centrifugal force effecting the components which separate into layers distributed across the radius of the rotor. Thus, radial separation is the maximum and minimum radius of the rotor separation path (Rmax, Rmin – see diagram). Retention of the radial separation distance in a range of rotors keeps resolution of components similar between rotors.

Scale rotors retain the radial separation distance while increasing the working volume. This allows an input volume from 20 ml to 200L, the latter in continuous flow mode.



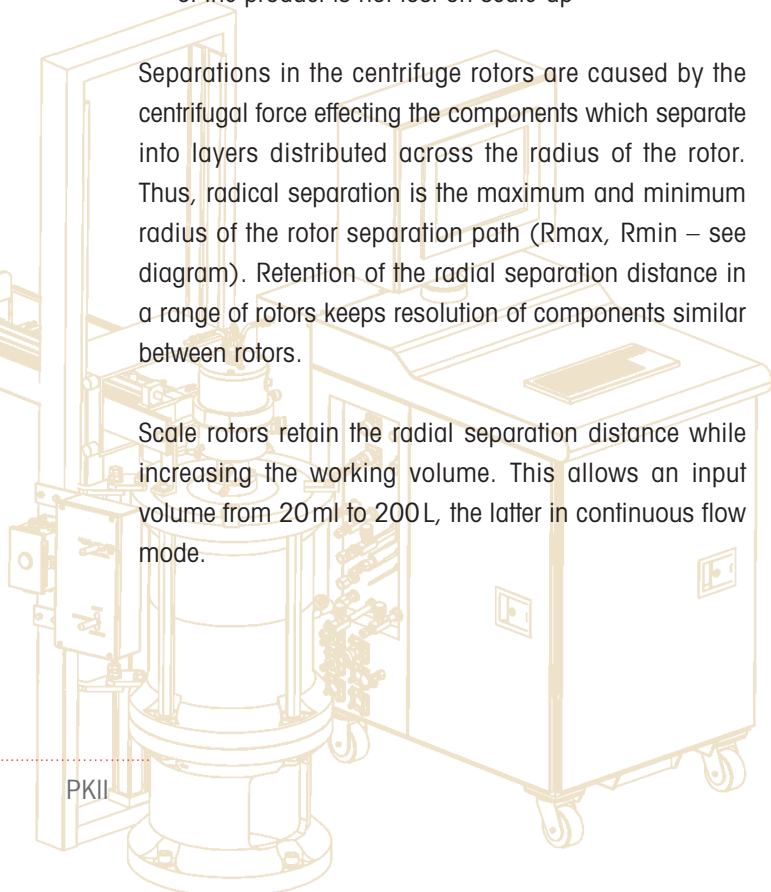
Calculate your scale-up easily

To run the same separation at a different scale, only the flow rate needs to be adjusted using the following formula:

$$\text{New flow rate} = \frac{\text{New Volume} \times \text{Flow Rate}}{\text{Old Volume}}$$

For example, using the 400 ml rotor running at 40,500 rpm with a gradient volume (1:1) of rotor volume at 1.2 L/hr. Then the flow rate for the production rotor (3200 ml) is:

$$\text{New flow rate} = (3200 \times 1.2) / 400 = 9.6 \text{ L/hr.}$$

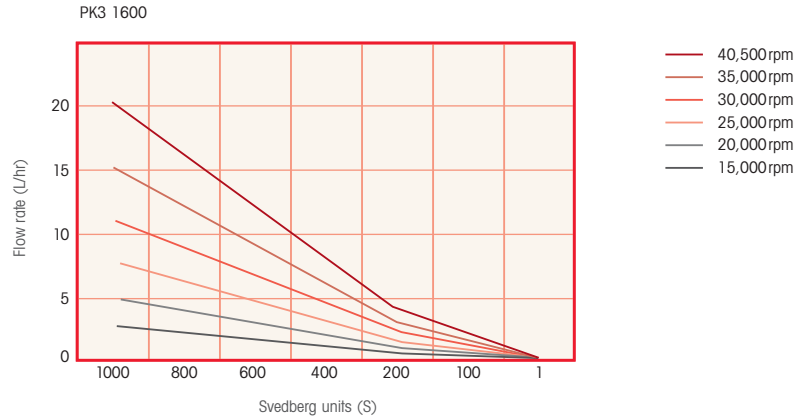


PKII

For a known virus, separation flow is simple to determine

EXAMPLE

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

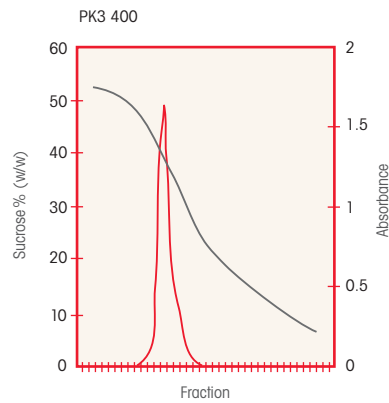


Scale-up by 8 fold produces the same product separation profile

RUN PARAMETERS

Volume 400 ml

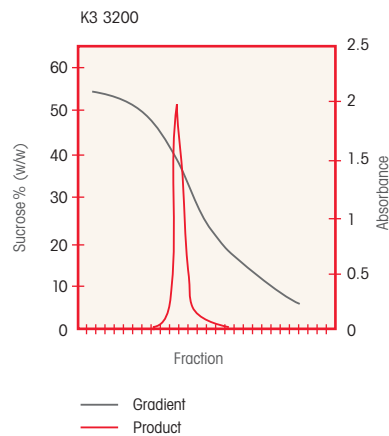
Rotor: PK3-400
 Gradient: Sucrose % (w/w)
 Density Gradient: 0–55% (w/w)
 Volume: 400 ml
 Process Volume: 10 L
 Speed: 40,500 rpm
 Flow Rate: 2.5 L/h
 Sedimentation Time: 2.5 min
 Particle: 130 nm, 1.9 g/cm³



RUN PARAMETERS

Volume 3.200 ml

Rotor: K3-3200
 Gradient: Sucrose % (w/w)
 Density Gradient: 0–55% (w/w)
 Volume: 3200 ml
 Process Volume: 80 L
 Speed: 40,500 rpm
 Flow Rate: 20 L/h
 Sedimentation Time: 2.5 min
 Particle: 130 nm, 1.19 g/cm³



KII Ultracentrifuge

THE CHOICE FOR INDUSTRIAL SCALE PRODUCTION

KII Ultracentrifuge for commercial vaccine manufacturing is fully validated to meet cGMP

The KII Centrifuge is the commercial centrifuge chosen for manufacturing of vaccines and other bioproducts

Large rotor volume allows high process volumes.

The control system has all the features expected from modern bioprocess equipment. Our over 30 years of experience in vaccine manufacture is brought together in the KII Ultracentrifuge and validation package. The KII features the following:

- > Reliable in full year production process
- > Efficient process purity
- > Unattended operation
- > Intuitive and simple operation
- > Closed loop operation
- > BL2+ environment compatible
- > Clean room class C/D compatible
- > Complete validation support



Implication of multiple step processes

In a typical purification and concentration scheme going from raw bioprocess material to a final formulation of pure biomaterial there are numerous steps involved. These include: initial clarification, filtration and chromatography steps and typically also buffer exchange steps. Using Alfa Wassermann's KII system does not only circumvent the use of these complex systems and costly column matrices but also increase the final yield by minimizing the steps involved in the purification process (see below).

Multi-step processes severely effect final yield

KII SYSTEM	CONVENTIONAL SYSTEM	YIELD/STEP	1	2	3	4	5
PRODUCTION – BIOREACTOR, 150 L	BIOREACTOR	yield 90%	90%	81%	73%	66%	60%
PURIFICATION – CLARIFICATION & SEPARATION USING THE KII CENTRIFUGE, 3.2 L ROTOR	CONCENTRATION	yield 80%	80%	64%	51%	41%	33%
FINAL FORMULATION, 10 L	DIAFILTRATION 1	yield 70%	70%	49%	34%	24%	17%
	COLUMN 1	yield 60%	60%	36%	22%	13%	8%
	DIAFILTRATION 2	yield 50%	50%	25%	13%	6%	3%
	COLUMN 2						
	CONCENTRATION/BUFFER EXCHANGE						
	FINAL FORMULATION						

Benefits of the KII Ultracentrifuge

- > No buffer exchange diafiltration needed
- > No pre-concentration of harvest material
- > Pre-clarification option with the K6 rotor
- > No filters or chromatography matrix to replace
- > Single step concentration and purification
- > Retains the infectivity of viruses in the low shear gradient environment
- > Yield up to 80%



Over 30 Years of Experience

COMMERCIAL MANUFACTURING

For over 30 years, the Alfa Wassermann centrifuge has been used for commercial purification vaccines.

In 1969, New Jersey based Electro-Nucleonics produced the first Model K centrifuge which was updated in the 1970's to the Model K Mark II.

The company joined the group of Alfa biotech in 1991 under the name of Schiapparelli Biosystems. They continued to manufacture in New Jersey, launching the Super G rotor extending the operational range from 35,000 rpm to 40,500 rpm.

In 1997, the Smart Console was introduced; a PC based computer to control the KII centrifuge.

In 2000, the company name was consolidated to the parent business name of Alfa Wassermann and continued improvement of the KII and validation package completing:

- > PC-HMI system with network capability
- > Linear scale rotors for experimental work

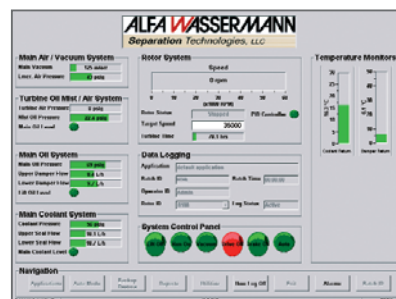


HMI (human-machine interface)

HMI is enclosed in an IP65 industrial housing with keyboard or touch screen input. HMI can be located remotely from the centrifuge in a separate room for BL2+ configuration or can be mounted on the centrifuge.

System Automation Features

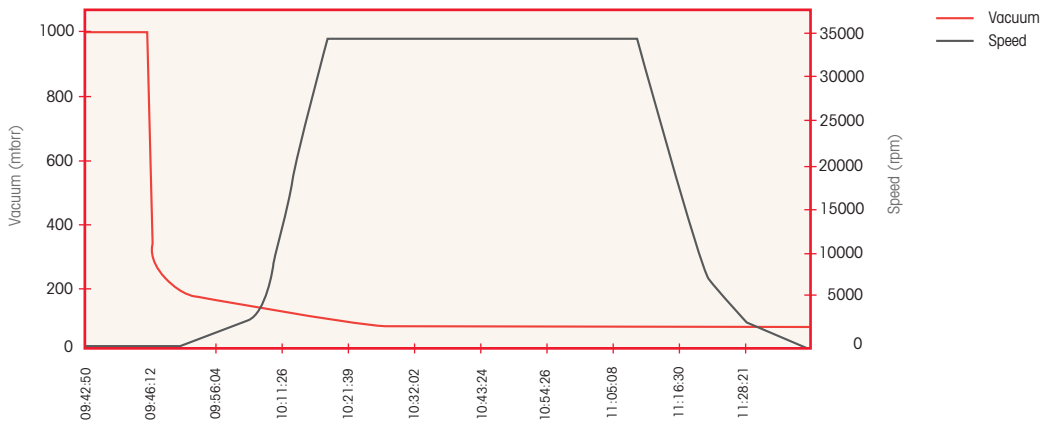
- > Windows 2000
- > PC-HMI for ease of use
- > Data acquisition to central computer
- > Password security
- > 21 CFR Part 11 compliance
- > GAMP compliance
- > Alarm system for shutdown and warning



Real time parameters and alarm display.

GMP Features

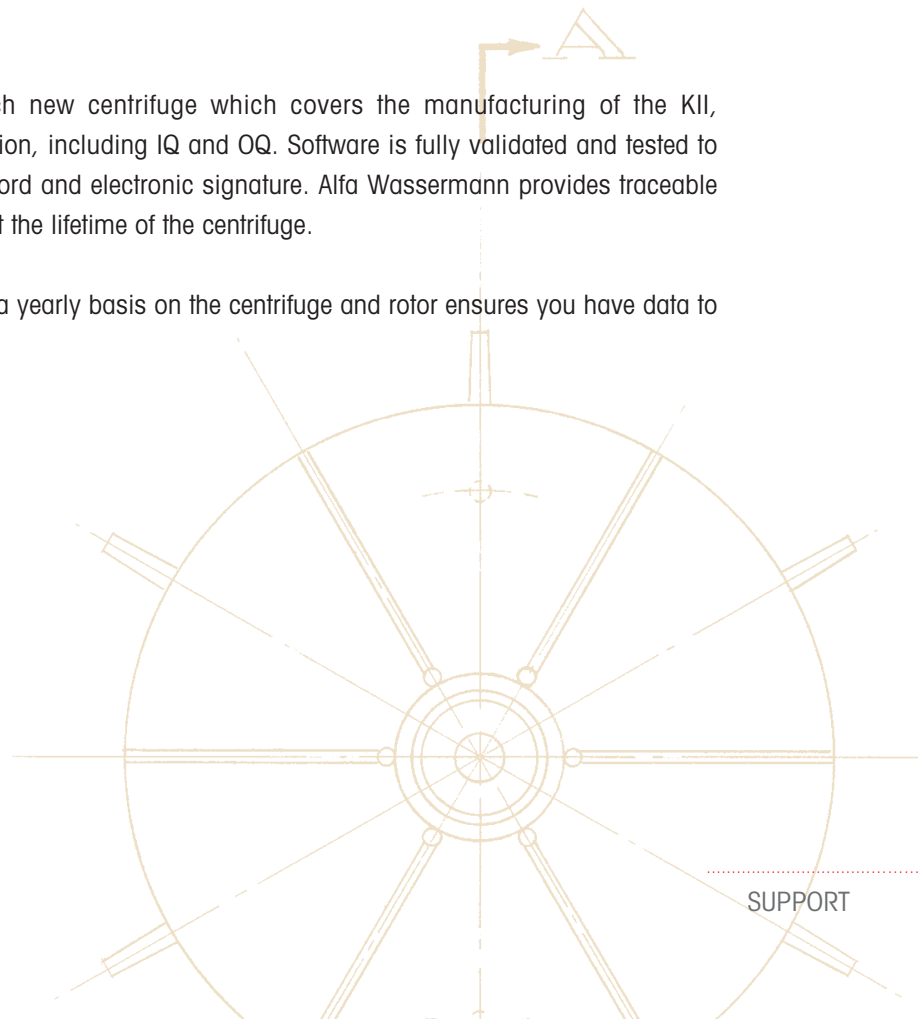
Data for each run is saved to a file which can be viewed as a table or plot. Comparison between batches for lot-to-lot reproductability is also possible on the system.



Alfa Wassermann Validation Support

We provide a full validation package for each new centrifuge which covers the manufacturing of the KII, software development, qualification and installation, including IQ and OQ. Software is fully validated and tested to meet 21 CFR Part 11 compliance – for batch record and electronic signature. Alfa Wassermann provides traceable material and conformance certification throughout the lifetime of the centrifuge.

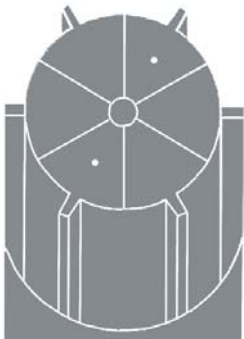
Routine service, maintenance and calibration on a yearly basis on the centrifuge and rotor ensures you have data to support GMP manufacture.



Application of the KII Centrifuge ...

VIRUS VACCINE AND VIRAL VECTOR MANUFACTURE

... to viral vaccine manufacture



K3 Rotor

INFLUENZA VIRUS VACCINE K3 ROTOR

System: KII
Rotor: K3 (3.2 L)
Flow rate: 20 L/h
Gradient: 0–55% (w/w) sucrose
Volume: 150 L
Capture rate: 95%
Recovery: 70%
Purification factor: x 50

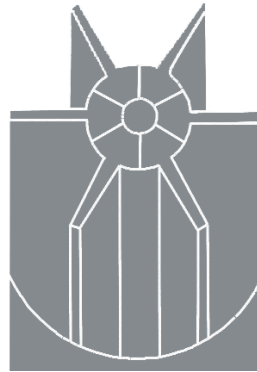
RABIES VIRUS VACCINE K3 ROTOR

System: KII
Rotor: K3 (3.2 L)
Flow rate: 16 L/h
Gradient: 0–65% (w/w) sucrose
Volume: 40 L
Capture rate: 95%
Recovery: 90%
Purification factor: x 90

... to viral vector manufacture

ADENOVIRUS VECTOR K3 ROTOR

System: PKII
Rotor: PK3 –1600
Flow rate: 10 L/h
Gradient: 0–40% Nycodenz
Volume: 20 L
Capture rate: 95%
Recovery: 70%
Purification factor: x 20



K5 Rotor

HEPATITIS B K5 ROTOR

System: KII
Rotor: K5
Flow rate: Batch
Gradient: 0–55% (w/w) sucrose
Volume: 5 L
Capture rate: 100%
Recovery: 85%
Purification factor: x 10

Protocols using the KII Centrifuges

EXAMPLES OF VIRUS ISOLATION PROTOCOLS

ADENOVIRUS – Adenoviridae
HEPATITIS B – Hepanaviridae
HBLV – Herpesviridae
INFLUENZA – Orthomyxoviridae
RABIES – Rhabdoviridae

NDV, MUMPS – Paramyxoviridae
RSV, MULV, MOMLV, AKRMLV – Retroviridae
JAPANESE ENCEPHALITIS – Flaviviridae
POLIO – Picornaviridae
VACCINIA – Poxviridae

ROTOR BODY	K ROTORS	PK ROTORS
Max. speed	40,500 rpm	40,500 rpm
Max. force	121,000 xg	121,000 xg
Max. radius	66 mm	66 mm
Residence length	76 cm	38 cm
Materials	Titanium, Noryl ®, PEEK	Titanium, Noryl ®, PEEK


CORE



K3 TYPE	K3	PK3
Min. force	100,000 xg	100,000 xg
Min. radius	55 mm	55 mm
Path length	11.5 mm	11.5 mm
Volume	3.2L	1.6, 0.8, 0.4 & 0.2L



K5 TYPE	K5 (batch gradients)
Min. force	38,500 xg
Min. radius	21 mm
Path length	45 mm
Volume	8.4L

CORE	K ROTORS	PK ROTORS
	K6 TYPE	K6
	Min. force	100,000 xg
	Min. radius	55 mm
	Path length	11 mm
Volume	3.2L	1.6, 0.8, 0.4 & 0.2L

BUILT-IN PRE-CLARIFIER

Max. force	53,900 xg	53,900 xg
Min. force	21,900 xg	21,900 xg
Max. radius	29 mm	29 mm
Min. radius	11.5 mm	11.5 mm
Volume	0.35L	0.17L

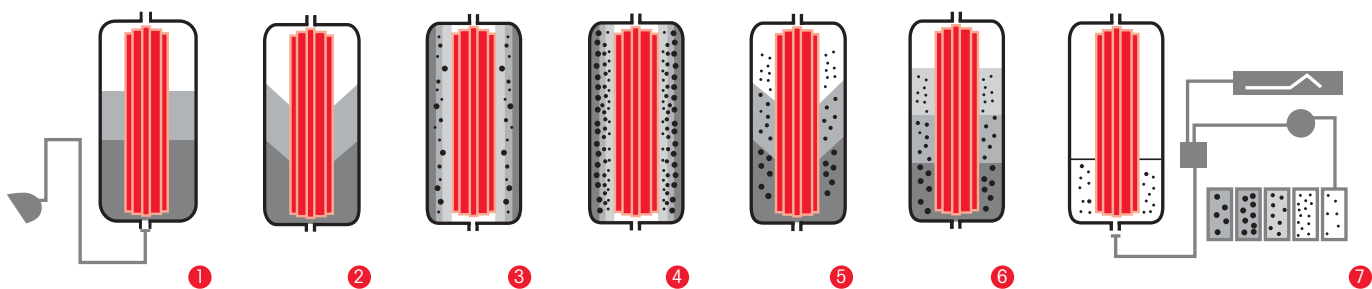


K10 TYPE	K10	PK10
Min. force	49,000 xg	49,000 xg
Min. radius	26.5 mm	26.5 mm
Path length	39.3 mm	39.3 mm
Volume	8.0L	4.0L



K11 TYPE	K11	PK11
Min. force	121,000 xg	121,000 xg
Min. radius	65.5 mm	65.5 mm
Path length	0.5 mm	0.5 mm
Volume	0.38L	0.19L

Reorienting gradient technique for simple separations



The density gradient is loaded into the rotor while it is at rest **1**. As the rotor is gradually accelerated, the gradient reorients itself vertically along the wall **2**. Sample fluid is now pumped into the rotor at one end on a continuous flow basis **3**. 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 **4**.

At the end of the run, the rotor is decelerated **5** and the gradient reorients itself to the original position without disturbing the particle bands **6**. The banded particles are now ready to be unloaded when the rotor is at rest **7**. Fractions are collected using a small peristaltic pump or air pressure.

Alfa Wassermann Ultracentrifuges for the Bioprocess Industry

- > All process volumes for high value therapeutics from 1 L to 200 L
- > Linear rotors 200–3200 ml
- > Air drive robustness industry standard
- > Alarm and data records

FEATURES

SPECIFICATIONS

System maximum speed and centrifugal force	40,500 rpm, 121,000 xg (Rmax)
Speed control	+/- 100 rpm
Rotor temperature accuracy	+ 4°C continuous flow, +/- 2°C batch
User interface	All run parameters, log on data, alarm status
Control system	Windows PC system
HMI	Touch screen and console top keyboard both IP65
Weight	KII: 1273 kg (2806 lbs.); PKII: 1204 kg (2654 lbs.)
Dimensions KII	297 (H) x 200 (W) x 130 (D) cm; 117 (H) x 78.8 (W) x 51 (D) inch
Dimensions PKII	220 (H) x 200 (W) x 130 (D) cm; 86 (H) x 78 (W) x 51 (D) inch
Power requirement	115/230V, 50/60 Hz 15 Amp (heat exchanger)
Drive unit warranty	4000 hours
System lifetime	Over 30 years
System warranty	One year
Compliance	CE, CSA, 21 CFR Part 11, GAMP

Design: www.schraegspur.com

ALFA WASSERMANN

www.AlfaWassermannUS.com

Alfa Wassermann, Inc.
4 Henderson Drive
West Caldwell, NJ 07006
USA
Tel. 1-800-220-4488
Fax 973-276-0383
separationtech@AWST.US

Alfa Wassermann, BV
Poppmolenlaan 24
3447 GK Woerden
The Netherlands
Tel. +31 348 487 300
Fax +31 348 433 000
office@AlfaWassermann.nl

An ISO 9001: 2000
Registered Company

