ALFA WASSERMANN

Separation Technologies

KII & PKII

Continuous Flow Ultracentrifuges

Density Gradient Ultracentrifugation systems for large scale and pilot scale downstream processing of viral vaccines and virus like particles.

Alfa Wassermann | Separation Technologies

Alfa Wassermann Separation Technologies (AWST) is the leader in ultracentrifugation solutions for process development and industrial scale manufacturing, exploiting over forty years of industry-leading continuous flow ultracentrifugation experience of its parent, Alfa Wassermann, Inc. (AWI).

The bioprocess industry has constantly relied on Alfa Wassermann continuous flow ultracentrifuges to efficiently and reliably separate viruses in the development and manufacture of life-saving vaccines and other biotherapies.

40 years of industry-leading continuous flow ultracentrifugation

History | Continuous Flow Ultracentrifuges

Alfa Wassermann continuous flow ultracentrifuges meet all the demands of cGMP manufacturing for viral vaccines and viral vector gene therapy products. Over forty years of Alfa Wassermann's experience has led to the development of a sophisticated and robust ultracentrifuge suitable for running upward of 3000 operational hours a year.

Continuous flow zonal ultracentrifugation was a major development of Dr N.G. Anderson and co-workers in the AEC-NIH Molecular Anatomy Program at Oak Ridge National Laboratories.

In 1967 this technique was made available commercially by Electro Nucleonics Inc (now Alfa Wassermann). Since then the worlds leading pharmaceutical manufacturers are utilizing the KII ultracentrifuge to produce purified Influenza vaccine on a large scale as well as Meningitis, Rabies, Hepatitis B and other vaccines.

PKII Ultracentrifuge

About | Drive Technology

Alfa Wassermann provides both the traditional Air Drive or an electric motor to provide the driving force to spin the rotor in the rotor chamber.

The Air Drive is traditionally used in high usage processes where robustness and durability are required.

The ‘e-drive’ electric motor has been developed for stand alone use with only an electrical supply required. The noise emissions of the e-drive KII are very low and therefore suitable for laboratory use.

System Control

A PLC or PC based control runs the proprietary AWST control interface console software. Critical lubrication and speed control systems are built in with alarms configured to protect the product and machine from harm.

eKII Ultracentrifuge

Control Interface

A clear windows based HMI allows for easy operator interaction. Input of data is via keyboard and touch screen. On screen visual and audible alarms enable unattended operation.

Monitoring Data

Speed
Vacuum
Lubrication Flow
Coolant Flow
Lubrication Level
Coolant Level
Refrigerator Temperature
Rotor Temperature
Monitoring Operations:
User ID
Password
Batch ID
Rotor ID
Batch Time
System Run Time
Rotor Run Time

Data Monitoring

Continuous data monitoring to a named batch file throughout the run can be reviewed on screen and is saved to the system before being transferred to a remote location. User configurable logging allows only process critical parameters to be trended. Batch data can be transferred to a process control unit or sent to a remote printer.

Validation

The system is fully validated for use in cGMP manufacturing processes and is the choice for vaccine manufacturers. The control console regulates and controls all necessary process fluids, safety features allow for unattended operation, password security enables compliance to 21 CFR Part 11 and GAMP.

Support Services

Full validation support is available from Alfa Wassermann during the Factory Acceptance Test, installation and onsite system validation. A full training program is supplied and service personnel provide a dedicated global support team to give service and maintenance support 7 days a week.

Rotors

Interchangeable rotors allow for a wide range of process volumes, flow rates and material compatibilities.

Speed	0 - 40 500 rpm
Centrifugal Force	38 500 * - 121 200 xg
Product Flow Rate	0 - 60 l/h
Rotor Volume Range	0.2 to 8 l
Process Volume	5 l to 200 l continuous flow*

* Dependant on core used

Scale Up & Scale Down of Processes

Density gradient ultracentrifugation allows concentration and purification in a single step, reducing the total number of process steps and process time and therefore increasing the overall yield and production capacity. Selection from a range of linear scalable rotor cores enables scale up and scale down of process parameters. The K3 linear scale cores retain the same separation path but differ in rotor volume, which allows for the exact same process purification to be achieved at production scale as well as at pilot scale volumes.

To start purification using the ultracentrifuge clarified harvest material can be processed directly, with no concentration or buffer exchange required. Bulk harvest can be processed without clarification using the integrated pre clarifier K6 rotor assembly.

The simple fluid path of the ultracentrifuge rotor creates a low shear environment which helps retain the viability of virus particles during downstream processing. Use of sucrose as a density gradient matrix is widely applied and cost effective for virus like particle purification and can easily be removed in subsequent processing.

KII Rotor Specification

The rotor assembly is composed of a tubular bowl and two end caps which are made of Titanium Alloy. These create the housing of the rotor assembly and contain within the rotor core. This assembly forms the six flow channels down which process material flows during operation.

Maximum Speed and Centrifugal Force: 40 500 rpm, 121 200 xg
Flow Rate Range: 0 l/h (batch) to 60 l/h
Process Volume: 5 l to 200 l continuous flow

Rotor Type	Application	Max. Force	Capacity with Core	Dimensions
 K3	For separation using isopycnic banding techniques with viral particles, virus like particles or 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	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 the 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 density.	At 40 500 rpm Rmax: 121 200 xg Rmin: 38 500 xg	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 or 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 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. The basis of separation is the difference in buoyant densities of the particles being separated.	At 40 500 rpm Rmax: 121 200 xg Rmin: 49 000 xg	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	0.38 liters	Diameter: Max: 130 mm / 5.2″ Min: 129.5 mm / 5.2″ Path Length: 0.5 mm / 0.02″

PKII Rotor Specification

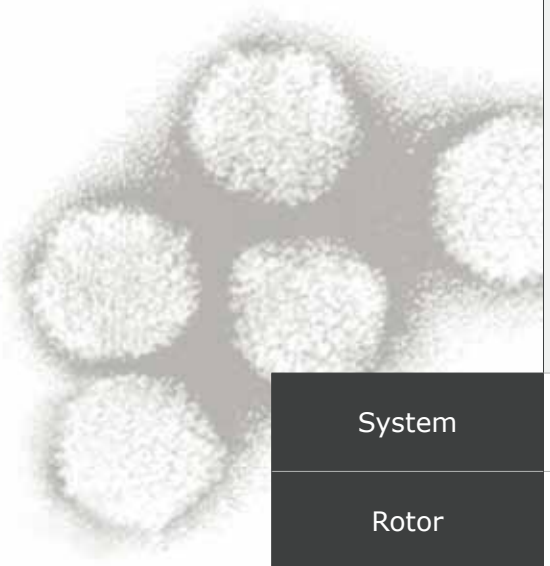
The PKII rotor specification have the same maximum / minimum radius as the KII rotors but are exactly half as long.

This means any separation developed on a PKII ultracentrifuge can be linear scaled up to a KII ultracentrifuge with only the change of process flow rate, using the same type rotor.

Rotor Type	Application	Max. Force	Capacity with Core	Dimensions
 PK3	For separation using isopycnic banding techniques with viral particles, virus like particles or 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	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 or 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	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 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. The 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	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	0.19 liters	Diameter: Max: 130 mm / 5.2″ Min: 129.5 mm / 5.2″ Path Length: 0.5 mm / 0.02″

Applications

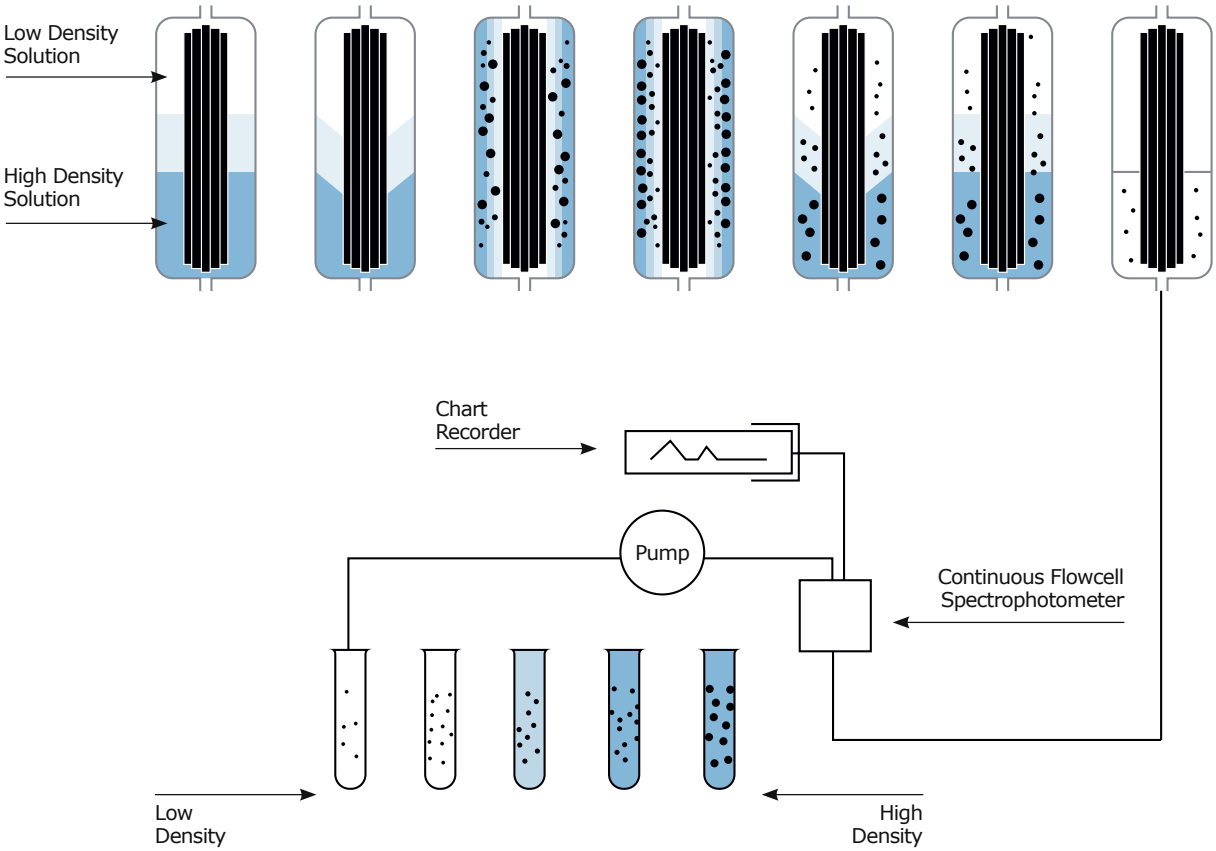
The ultracentrifugation process typically uses density gradient continuous flow ultracentrifuge for the purification and concentration of virus particles. The table below describes the general conditions for separation and purification of a range of viral products.



	Influenza Vaccine	Rabies Vaccine	Hepatitis B Vaccine	Adenovirus Vector
System	KII	KII	KII	PKII
Rotor	K3 3.2 l	K3 3.2 l	K5 8.4 l	PK3 1.6 l
Flow Rate	20 l/h	16 l/h	Batch Operation	10 l/h
Gradient	0-55% (w/w) sucrose	0-65% (w/w) sucrose	0-55% (w/w) sucrose	0-40% (w/w) Nycodenz®
Volume	150 l	40 l	5 l	20 l
Capture	95%	95%	100%	95%
Recovery	70%	90%	80%	70%
Purification factor	x 50	x 90	x 10	x 20

Zonal Density Gradient Reorientation

Step	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.



System Technical Data

Parameter	Specification
Rotor Material	Titanium Alloy
Core Material	Noryl®, PEEK or Titanium
Drive Technology	Electric Motor or Air Drive
Process Flow Rates & Pressures	Up to 60 l/h @ 1 bar max.
Process Temperatures	4 to 30°C +/- 2°C
Process Volumes	200 liters per run
Maximum Speed	40 500 rpm +/- 100 rpm
Maximum Centrifugal Force	121 200 xg
Process Connections	3A Sanitary Fittings
Material (process wetted)	316LSS, Titanium, Noryl®, Teflon, 440C SS
Gaskets	USP Class VI
Lubricants	WFI & Pharmaceutical Grade H1 Hydraulic Oil
Noise emissions	Compliant with CE; suitable for use in laboratory environments
Control Panel Specifications	IP65 HMI with touch screen for access to; virtual controls, LED alarms, on screen gauges and trending. The display units are configurable.
Interface Language	English, French, German, Italian, Spanish
Regulatory Compliances	21 CFR part 11, GAMP, cGMP
Manufacturing Compliances	CE, ISO 13485 Registered Company
Rotor Handling	System integrated lift and rotor cart for storage
Warranty	System 1 year including rotor

Installation Requirements

	e-drive	Air Drive
Models Available	eKII and ePKII	KII and PKII
Process Air	N/A	2.83 m^3/min
Process Cooling	Integrated	4.5 °C, 4 l/min
Electrical Supply	32A, 1ph 230V	15A, 1ph 230V
Environmental Conditions	0-40°C, RH 85%	0-40°C, RH 85%
Clean Room Classifications	Class B,C,D	Class B,C,D
Bio-containment	Up to BL3	Up to BL3
System Footprint	900 x 1650 (W x D)	2140 x 1330 (W x D)
Height Requirements	295 cm (ePKII 220 cm)	295 cm (PKII 220 cm)
System Weight	1270 kg (ePKII 1204 kg)	1270 kg (PKII 1204 kg)

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Ref. SM307