بسم الله الرحمن الرحيم

MEGBI Vaccine Pilot Plant – 2nd Project Report

(for project period Feb 2013 – Dec 2013)

تصميم لمحطة انتاج لقاح HBsAg اولية

- Downstream processing elements for Hepatitis B DNA vaccine production process
- Process Chromatographic Purification Device
- Bioreactor Automation

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مركز أبحاث الشرق الأوسط للجينات والتقنية البيولوجية

رأسنحاش – قضاء البترون– لبنان

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http://www.aecenar.com/institutes/megbi

الشركة اللبنانية الالمانية للبيوتكنولوجيا

رقم ٣٩٩ في سجل التجاري بيروت مسجل في تاريخ ٢٠٠٩/٥/٢٨

رأسنحاش – قضاء البترون– لبنان

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المضمون

1 مدخل

This document is the base for MEGBI activity in 2013. From 2008 – 2011 MEGBI Genetic Engineering S2 lab was built.

2012 the MEGBI biotechnological upstream downstream chain was initialized. Also a bioreactor was started to be manufactured.

In 2013 there was worked on a pilot plant facility for producing recombinant Hepatitis B Surface Antigen Vaccine.

In 2014 it is planned to develop a chromatographic purification device for production scale similar to AKTA process.

2 Project Management

2.1 Time plan and Costs

2.1.1 Phase I

	initiating facility	Establishing Training Courses	Research on H5N1 vaccine
Time period:	Sep 08 - Aug 09	Sep 09 - Aug 10	Sep 10 - Aug 11
Total costs:			
Devices	20,000.00 €	12,000.00 €	1,000.00 €
Material	1,000.00 €	3,000.00 €	2,500.00 €
Personnel	1 Person	5 Persons (Students) + Supervisor	1 Person + Supervisor
	35,000.00 €	15,000.00 €	
Renting rooms	1,000.00 €	6,000.00 €	6,000.00 €

Total costs:
102,500.00 €

Time period:
3 years

2.1.2 Phase II

MEGBI 2012/2013 – Biotech chain

Rent: 4200 USD

Personnal costs: 500 USD

Material costs: 1000 USD

Offers:

Bioreactor (100 L) 100.000 EUR

Akta purifiying system 35.000 EUR

2.1.3 Phase III

…tdb…

3 Basics

3.1 بعض الاساسيات الاضافية بالنسبة للبيورياكتور و فحص دواء جديد بالحيوانات

3.1.1 Project Overview

In the figure below, you can see the drug development process

Source: PhRMA

3.1.2 Phase I: identification of drug candidate

In our case: H5N1 subunit vaccine

3.1.3 Phase II:Preclinical Tests [1]

Before a medicament is approved, the quality, safety and efficacy of any product must be demonstrated. Demonstration of conformance to these requirements is largely attained by undertaking clinical trials. However, preliminary data, especially safety data, must be obtained prior to the drug`s administration to human volunteers. These safety data, has to be explored in preclinical pharmacological and toxicological experiments accomplished in animals. Such preclinical studies can take up to 3 years to complete, and at a cost of anywhere between US$10 million and US$30 million. On average, about 10% of potential new drugs survive preclinical tests.

Provided guidelines demonstrates the range of tests during preclinical studies.

3.1.3.1 Pharmacokinetics and Pharmacodynamics

Pharmacokinetics relates to the fate of a drug in the body, particularly its ADME (**A**bsorption, **D**istribution, **M**etabolism, and **E**xcretion). The results of such studies not only help to identify any toxic effects, but also point to the most appropriate method of drug administration, as well as the most likely effective dosage regime to employ. Generally, ADME are undertaken in two species,

[1]Mostly from [Walsh 2007]

usually rats and dogs, in each case males and females. Sometimes it is necessary also using primates.

Pharmacodynamic studies deal more specifically with how the drug brings about its characteristic effects. Focus in such studies is often placed upon how a drug interacts with a cell/organ type.

3.1.4 Bioavailability and Bioequivalence

Bioavailability and bioequivalence are also usually assessed in animals. The Bioavailability of a drug which is parenteral (e.g. by injection) delivered is virtually 100%. If the drug is delivered by mouth, in most cases bioavailability is given at or near 0%.

Bioequivalence studies come into play if any change in product production/delivery systems was being contemplated. These studies would seek to identify whether such modifications still yield a product equivalent to the original one in terms of safety and efficacy. Modifications could include an altered formulation or method of administration, dosage regimes, etc.

3.1.5 Protein pharmacokinetics

A condition to make pharmacokinetic/pharmacodynamic studies is the availability of an adequate selective and sensitive assay. The assay have to describe the characteristics of the therapeutic protein in the presence of a complex soup of "contaminant" molecules, how it is given in body (e.g. tissue extracts or body fluids). The specific characteristics of those drugs can be realized either via immunoassay or bioassay.

The macromolecular structure of drugs and the fact that relatively minor structural alterations can potentially have a major influence upon bioactivity are often complicating factors. For example, an immunoassay may be blind to the oxidation of an amino acid residue, or very limited proteolytic processing, although such events can activate or decrease bioactivity. Pharmacokinetic and indeed pharmacodynamic characteristics of therapeutic proteins can be rendered complicated by a number of factors, including:

The presence of serum-binding proteins. Some biopharmaceuticals (including insulin-like growth factor (IFG)) are notable in that the blood contains proteins that specifically bind them. Such binding proteins can function naturally as transporters or activators, and binding can affect characteristics such as serum elimination rates.

Immunogenicity. Many therapeutic proteins are potentially immunogenic when administered to humans. However, human proteins can also be potentially immunogenic. Antibodies raised in this way can bind the therapeutic protein, neutralizing its activity and/or affecting its serum half-life.

Sugar profile of glycoproteins. The exact glycosylation pattern which is caused by therapeutic glycoproteins in different eukaryotic expression systems, can influence protein activity and stability in vivo.

3.1.6 Toxicity studies

Acute toxicity is usually assessed by administration of a single high dose of the test drug to rodents. Both rats and mice (male and female) are usually employed. The calculation of the LD_{50} value is outdated. Nowadays, calculation of the approximate lethal dose is sufficient. Chronic toxicity studies require large numbers of animals. Most chronic toxicity studies demand daily administration of the test drug at three different dosage levels. The highest level should ideally induce a mild but observable toxic effect, whereas the lowest level should not induce any ill effects.

During this process all animals are subjected to routine clinical examination, and periodic analyses (e.g. blood, urine tests).

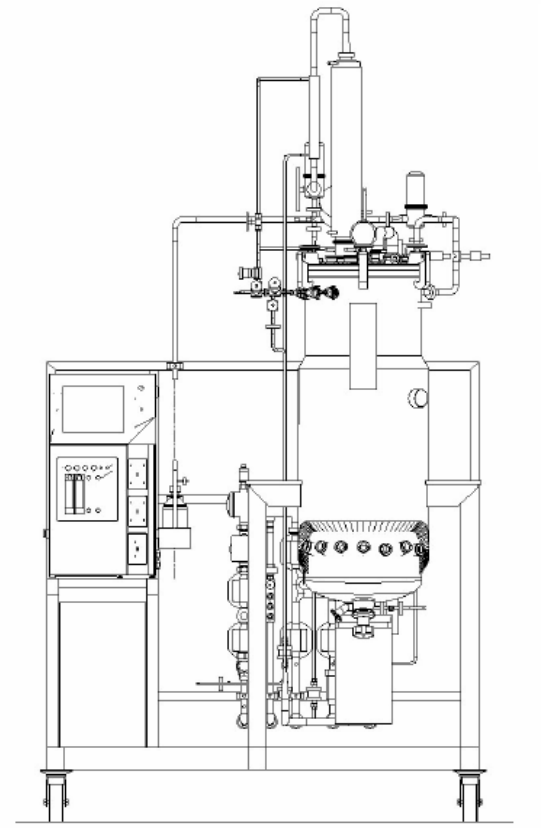
Reproductive toxicity and teratogenictiy. Fertility studies aim to assess the nature of any effect of the substance on male or female reproductive function. The drug is administered to males for at least 60 days. Females are dosed for at least 14 days before they are mated. Specific tests carried out include assessment of male spermatogenesis and female follicular development, as well as fertilization, implantation and early foetal development. A teratogen is any substance/agent that can induce foetal developmental abnormalities. Examples include alcohol, radiation and some viruses.

Mutagenicity. Mutagenicity tests aim to determine whether the proposed drug is capable of inducing DNA damage, either by inducing alterations in chromosomal structure or by promoting changes in nucleotide base sequence. These tests are usually carried out in vitro and in vivo, often using both prokaryotic and eukaryotic organisms.

For phase II: upstream – downstream without clean room

3.1.7 Phase III

Production for clinical tests (upstream – downstream – pilot site with clean room)



Bioreaktor

Pilot System 130 L

For Cell Application

3.2 لقاح التهاب الكبد الفيروسي ب (HBsAg vaccine)

ويضمن هذا **اللقاح** الوقاية من عدوى التهاب الكبد الفيروسي ب .واللقاح يحتوي على واحد من البروتينات المغلفة للفيروس، وهو المستضد الموجود على السطح الخارجي لفيروس بي .(HBsAg) وهي مصنعة بواسطة خلايا فطر الخميرة، المدرج بداخلها الشفرة الوراثية للـ .HBsAg مقرر مكون من (3)

جرعات، الثانية تؤخذ بعد شهر واحد على الأقل من الجرعة الأولى والثالثة تؤخذ بعد الجرعة الأولى بستة أشهر.[1] بعد ذلك ينشئ جهاز المناعة أجسام مضادة ل HBsAg في الدم.
وتعرف الأجسام المضادة لـ *HBsAg* ب *anti-HBsAg* هذه الأجسام المضادة وذاكرة جهاز المناعة يوفر الحصانة ضد العدوى بالتهاب الكبد الفيروسي ب.[2] وأول لقاح أصبح متاح في عام 1981.

وهناك مجموعة من اللقاحات المتوفرة في السوق. في الوقت الحاضر لقاحات مصنعة بواسطة تكنولوجيا الهندسة الوراثية]][[متوفرة، وهو ما يعني أنها تنتج عن طريق إدخال الوحدة الوراثية لالتهاب الكبد الفيروسي البائي في الخميرة المعروفة حيث يتم زرعها، وتجميعها، وتنقيتها. لا يمكن أن تحدث عدوى التهاب الكبد الفيروسي بي نتيجة أخذ اللقاح.

العلامات التجارية العروفة والمتاحة هي Engerix-B (GSK), Elovac B (Human Biologicals Institute, A division of Indian Immunologicals Limited), Genevac B (Serum Institute), Shanvac Bالخ. كل هذه التطعيمات تؤخذ في العضل.

HBsAg vaccine production 3.3

Table 1. Commercially available yeast-derived vaccines (selection)

Product	Trade name	Company	Recombinant host organism
HBV vaccine			
	AgB	Laboratorio Pablo Cassará; (LPC)	*H. polymorpha*
	Biovac-B	Wockhardt	*H. polymorpha*
	ButanNG	Instituto Butantan/ N.G. Biotecnologia	*H. polymorpha*
	Engerix B	GlaxoSmithKline	*S. cerevisiae*
	Enivac-HB	Panacea	*P. pastoris*
	Gene Vac-B	Serum Institute of India	*H. polymorpha*
	Hepavax-Gene	Green Cross Vaccine	*H. polymorpha*
	Probivac	Probimed	*H. polymorpha*
	Recombivax	Merck and Co., Inc.	*S. cerevisiae*
	Shanvac-B	Shanta	*P. pastoris*
Multivalent vaccines (Including rHBsAg)			
	Infanrixhexa HB+HIB+Polio+DTP	GlaxoSmithKline	*S. cerevisiae* (HBsAg)
	Tritanrix HB+HIB+DTP	GlaxoSmithKline	*S. cerevisiae* (HBsAg)
HPV vaccine	Gardasil	MSD	*S. cerevisiae*

Upstream Processing 3.3.1

عمليات المعالجة عند المنبع

From

http://www.biopharminternational.com/biopharm/Downstream+Processing/Recombinant-Vaccine-Production-in-Yeast/ArticlesStandard/Article/detail/485189

Recombinant Vaccine Production in Yeast.

اللقاح المأشوب والمنتج في الخميرة

Methylotrophic yeast provide balanced production of the membrane and protein components of a recombinant viral particle.

خميرة الـ (Methylotrophic تزودنا بمنتج متوازن من الغشاء والمكونات البروتينية من جزيئات المصدر الفيروسي المدروس

Product-containing cells are usually generated by a two fermenter cascade, consisting of a 5-L seed fermenter used to inoculate the 50-L main fermenter. The whole fermentation process, starting from a single vial from the working cell bank, yields a biomass of more than 10 g dry cell weight per liter in 55 hours. The production fermentation is carried out in synthetic medium feeding glycerol in the first phase, and a mixture of glycerol and methanol in the second phase.[7]

المنتج يحتوي على خلايا والتي تنتج عادة من جرآء 5-L seed) المخمرة والتي تُستخدم لتطعيم الـــ --------؟

عملية التخمر والتي تحتوي على (

كل عمليات التخمر تبدا من فيروس مفرد من خزان الخلايا الفعالة لتنتج كتلة حيوية أكثر من 10 غرام خلايا جافة في الليتر الواحد خلال 55 ساعة .

الانتاج المخمر يكتمل في اصناع وسط مغذي غليسرولي في الطور الاول , ومزيج من الغليسرول والميثانول في الطور الثاني.

Remark: There should be used a one-way bioreactor for the first project period.

3.3.2 Downstream Processing

عمليات المعالجة عند المصب

The vaccine particles are harvested and cells are disrupted by a sequence of ion exchange, ultra filtration, and gel filtration steps.[7] The purified HBsAg particles are formulated by adsorption to an aluminum hydroxide adjuvant and addition of a preservative. A single adult dose containing 10 or 20 g of rHBsAg may be administered in three single injections at 0, 1, and 6 months.

يتم جمع جزيئات اللقاح والخلايا تتمزق من خلال تعاقب عدة عمليات كـــ عملية تبادل شوارد , عمليات فوق الفلترة , ومراحل فلترة باستخدام الجل .

تنقية جزيئات المستضد الموجود على السطح الخارجي لفيروس بي وتنتج من خلال عملية تسمى الادمصاص أو الامتزاز على هيدروكسيد الالمنيوم

References

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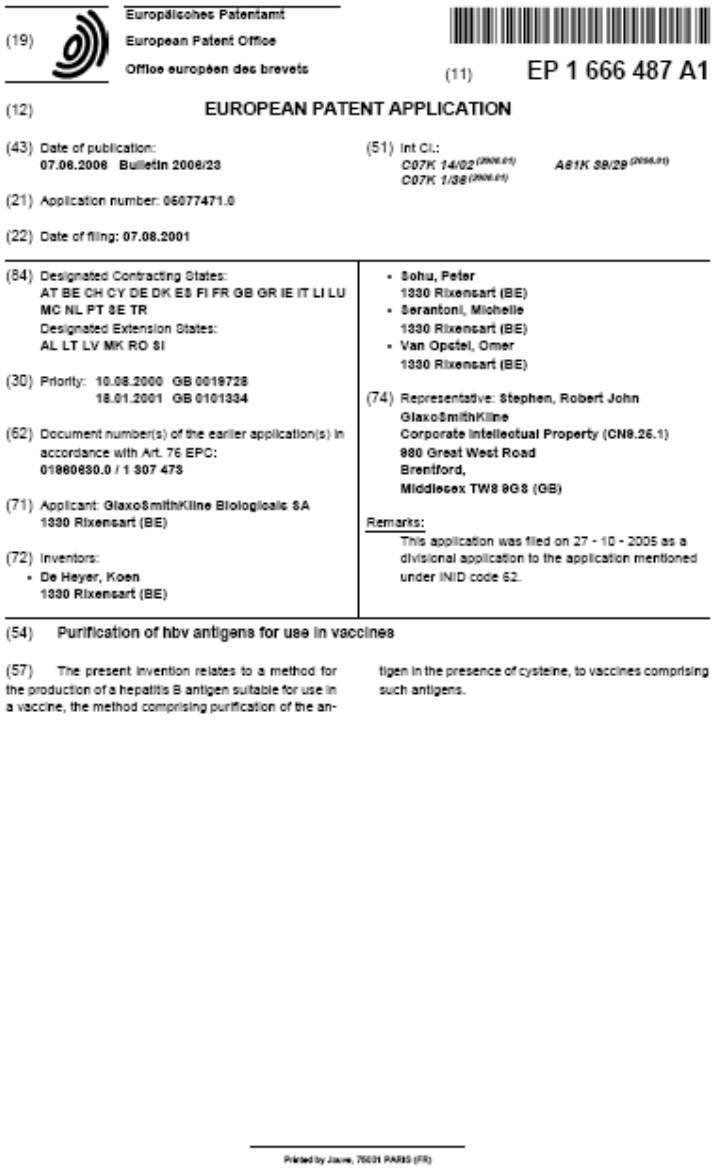
7. Brocke P, et al. Recombinant hepatitis B vaccines: disease characterization and vaccine production. Gellissen G, editors. In: Production of recombinant proteins. Weinheim, Germany: Wiley VCH;2000, pp. 319–359.

3.4 Engerix B (rHBsAg produced in S. cerevisiae)[2]



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C07K 1/36 (2006.01)

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Remarks:
This application was filed on 27 - 10 - 2005 as a divisional application to the application mentioned under INID code 62.

(54) Purification of hbv antigens for use in vaccines

(57) The present invention relates to a method for the production of a hepatitis B antigen suitable for use in a vaccine, the method comprising purification of the antigen in the presence of cysteine, to vaccines comprising such antigens.

Printed by Jouve, 75001 PARIS (FR)

The present invention relates to a method for the production of a hepatitis B antigen suitable for use in a vaccine, the method comprising purification of the antigen in the presence of cysteine, to vaccines comprising such antigens.

الابتكار الحالي يتعلق بطرق انتاج مستضد اللتهاب الكبد ب المناسب للاستخدام كاللقاح والطرق تتضمن تنقية المستضد خلال وجوده بسيستين ,لتلقيح يتضمن مثل هذا المستضد .

Claims:

1. A method for producing a stable, immunogenic hepatitis B vaccine without thiomersal suitable for human use, the method comprising:

[2] [Patent Engerix B]

2. الطريقة لإنتاج لقاح مناعي لالتهاب الكبد بدون -------؟ مناسب للاستخدام البشري والطريقة تتضمن

(a) expressing the hepatitis B surface antigen (HBsAg) as a recombinant protein in Saccharomyces cerevisiae;

يعبر عن مستضد التهاب الكبد بـــ (---------) كـــ بروتين مأشوب في (خميرة الـــ)

(b) processing the yeast cells to provide a crude antigen preparation;

معالجة خلايا الخميرة لتعطي طليعة المستضد

(c) subjecting the crude antigen preparation to gel permeation chromatography, wherein the elution buffer used in the gel permeation chromatography does not contain thiomersal, thereby producing an antigen-containing eluent;

خضوع عملية تحضير طليعة المستضد للكروماتوغرافيا النفوذه للجل , والذي من خلاله محلول التصفية المستخدم في الكرماتوغرافيا النفوذه للجل لايحتوي --------- وبتلك الوسيلة يتم انتاج محلول يحتوي المستضد .

(d) subjecting the antigen-containing eluant from step (c) to ion exchange chromatography;

وبعالجة المحلول الحاوي على المستضد في المرحلة سي بعملية كروماتوغرافيا التبادل الشاردي

(e) adding cysteine to the antigen-containing eluant obtained after step (d);

اضافة السيستئين للمحلول الحاوي على المستضد والناتج عن المرحلة دي

(f) subjecting the preparation from step (e) to ultracentrifugation, thereby obtaining a purified HBsAg;

معالجة المحلول الناتج عن المرحلة السابقة لعملية الطرد المركزي , وبالتالي نحصل على مستضد التهاب الكبد النقي

(g) combining the purified HBsAg with a pharmaceutically acceptable excipient to produce a stable, immunogenic hepatitis B vaccine suitable for human use;and wherein no thiomersal is added to the resulting vaccine.

ربط المستضد النقي مع السواغ المقبول صيدلانيا لإنتاج لقاح مناعي ثابت لالتهاب الكبد نمط ب صالح للاستخدام البشري والذي يتم به عدم اضافة ---كبريتي للقاح الناتج .

2. A method according to claim 1, wherein the cysteine is added to a final concentration of between 1 and 10 mM.

الطريقة حسب الاجراء الاول , حيث تمت اضافة السيستين للتراكيز النهائي من 1 الى 10 م م

3. A method according to claim 1, wherein the cysteine is added to a final concentration of about 2 mM.

الطريقة حسب الاجراء الاول حيث السيستين قد تمت اضافته للتراكيز النهائية بحوالي 2 م م

4. A method according to claim 1, wherein the ultracentrifugation is cesium-chloride ultracentrifugation.

الطريقة حسب الاجراء الاول , حيث عملية الطرد المركزي تكون طرد مركزي بـــ كلوريد السيزيوم

5. A method according to claim 1 which further comprises an ion-exchange chromatography step after gel permeation (c) and before ultracentrifugation (d).

الطريقة حسب الاجراء الاول والتي تشكل لحد بعيد مرحلة كروموتوغرافيا التبادل الشاردي بعد نفوذية الجل في المرحلة سي وقبل عملة الطرد المركزي

6. A method according to claim 5, wherein the ion-exchange chromatography is anion-exchange chromatography.

الطريقة حسب الاجراء 5 حيث كروماتوغرافيا التبادل الشاردي تكون كروماتوغرافيا تبادل غير شاردي

United states

patent application publication

نشر اكتشاف مسجل

purification of HBV antigens for use vaccines

تنقية مستضد التهاب الكبد لاستخدامه كاللقاح

correspondence address glaxosmithkline

عنوان المراسلة لاسم الشركة

ABSTRACT

The present invention to a method for the production of a hepatitis B antigen suitable for use in a vaccine , the method comprising purification of the antigen in the presence of cysteine to vaccines comprising such antigens.

(19) **United States**

(12) **Patent Application Publication**

DE-HEYDER et al.

(10) **Pub. No.: US 2009/0123496 A1**

(43) **Pub. Date: May 14, 2009**

(54) **PURIFICATION OF HBV ANTIGENS FOR USE IN VACCINES**

(75) Inventors: **Koen DE-HEYDER**, Rixensart (BE); **Peter Schu**, Rixensart (BE); **Michelle Serantoni**, Rixensart (BE); **Omer Van-Opstal**, Rixensart (BE)

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(21) Appl. No.: **12/342,220**

(22) Filed: **Dec. 23, 2008**

Related U.S. Application Data

(63) Continuation of application No. 10/344,211, filed on Jul. 18, 2003, now abandoned, filed as application No. PCT/EP01/09100 on Aug. 7, 2001.

(30) **Foreign Application Priority Data**

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Jan. 18, 2001 (GB) 0101334.1

Publication Classification

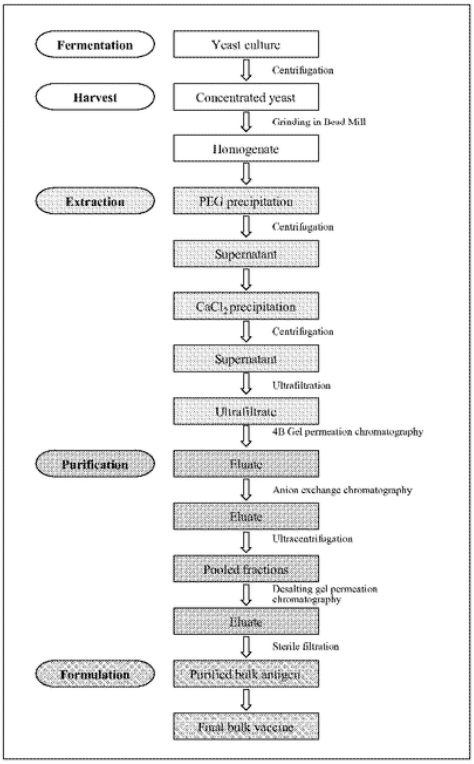
(51) **Int. Cl.**
A61K 39/29 (2006.01)

(52) **U.S. Cl.** **424/227.1**

(57) **ABSTRACT**

The present invention relates to a method for the production of a hepatitis B antigen suitable for use in a vaccine, the method comprising purification of the antigen in the presence of cysteine, to vaccines comprising such antigens.

Flow diagram of the thiomersal free production process for Engereix B™



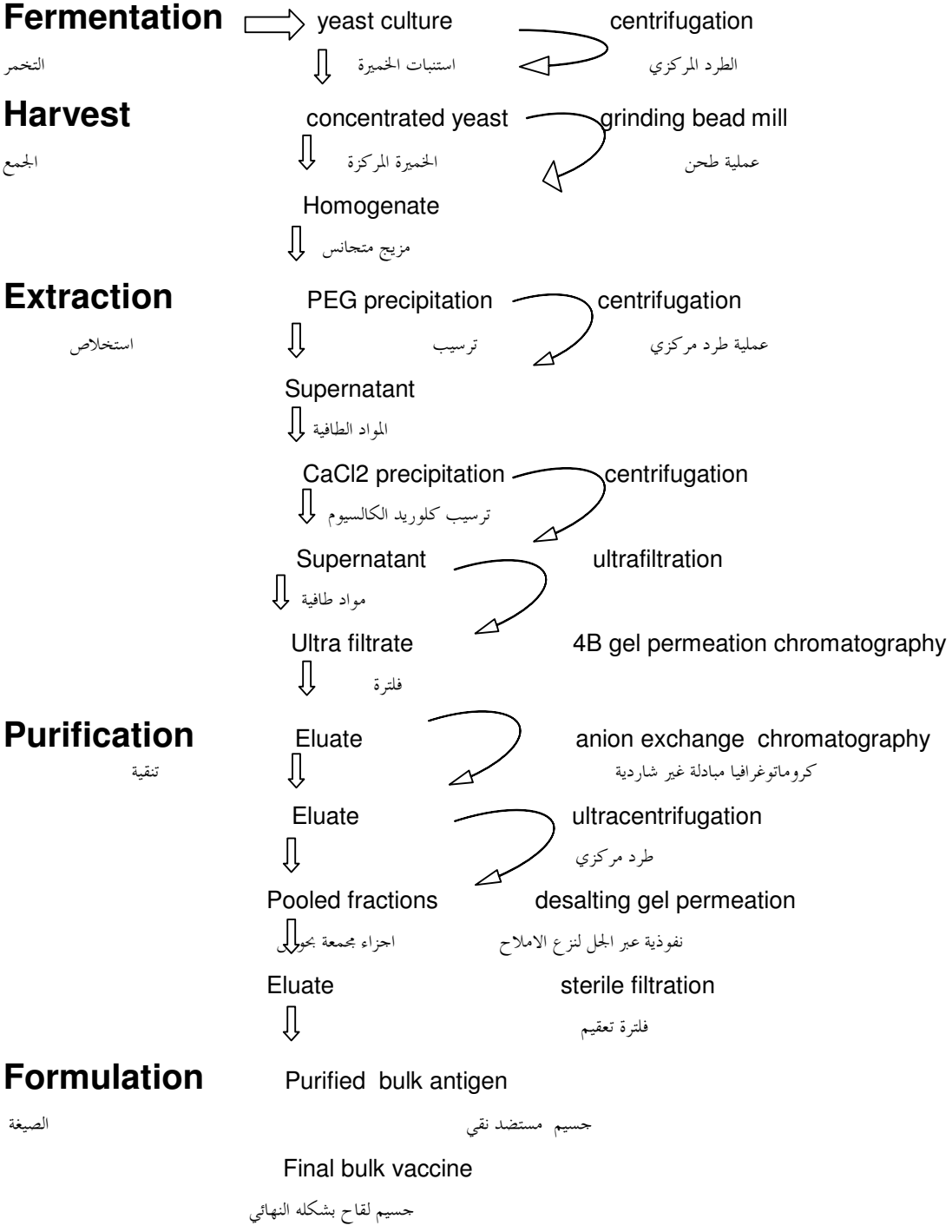
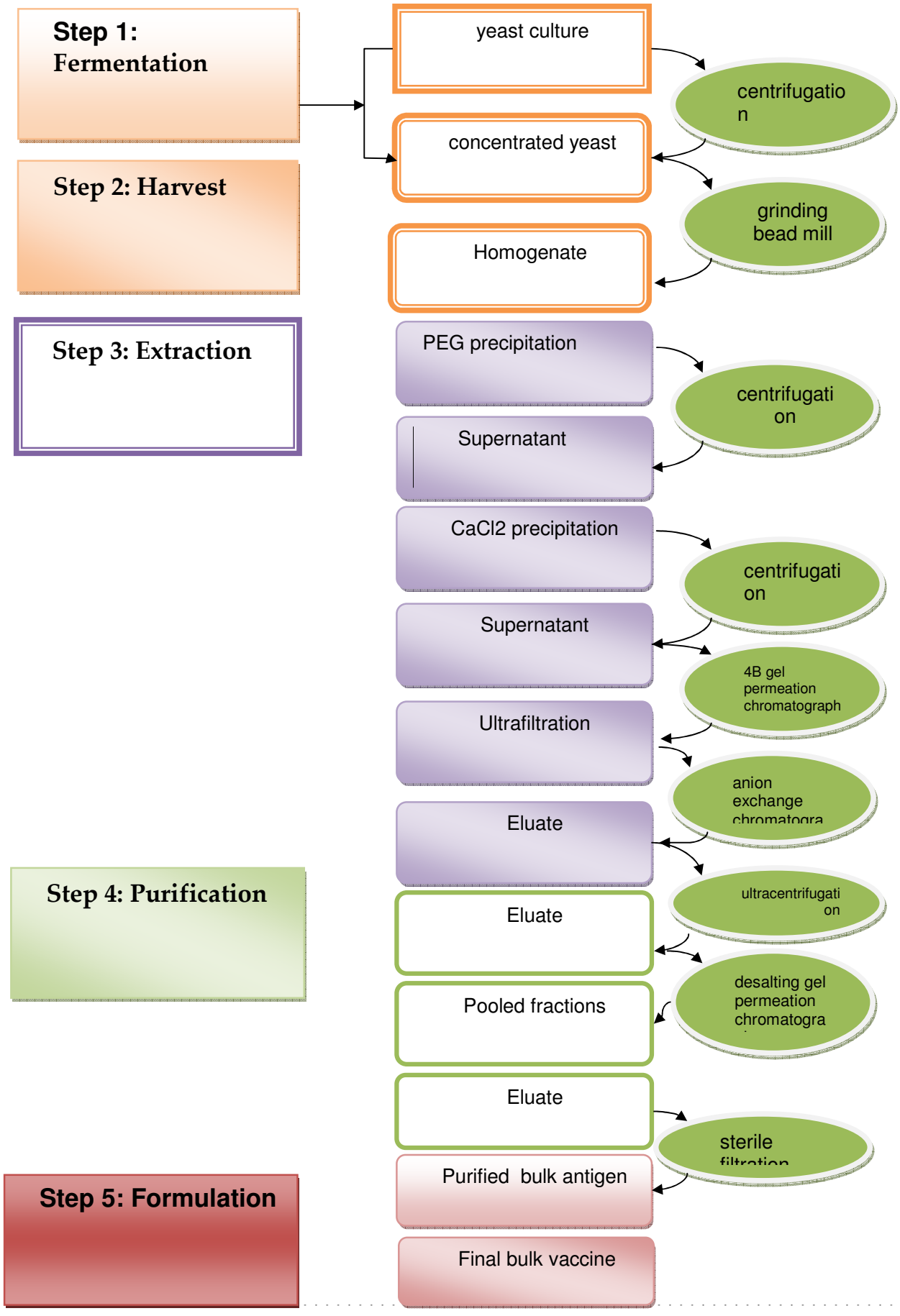

Fermentation
التخمر
yeast culture
استنبات الخميرة
centrifugation
الطرد المركزي
Harvest
الجمع
concentrated yeast
الخميرة المركزة
grinding bead mill
عملية طحن
Homogenate
مزيج متجانس
Extraction
استخلاص
PEG precipitation
ترسيب
centrifugation
عملية طرد مركزي
Supernatant
المواد الطافية
CaCl2 precipitation
ترسيب كلوريد الكالسيوم
centrifugation
Supernatant
مواد طافية
ultrafiltration
Ultra filtrate
فلترة
4B gel permeation chromatography
Purification
تنقية
Eluate
anion exchange chromatography
كروماتوغرافيا مبادلة غير شاردية
Eluate
ultracentrifugation
طرد مركزي
Pooled fractions
اجزاء مجمعة بحوض
desalting gel permeation
نفوذية عبر الجل لنزع الاملاح
Eluate
sterile filtration
فلترة تعقيم
Formulation
الصيغة
Purified bulk antigen
جسيم مستضد نقي
Final bulk vaccine
جسيم لقاح بشكله النهائي

Figure 1: flow diagram of the thiomersal free production process for Engerex B ™



3.4.1 Fermentation

European Patent 0199698

Produced in yeast

Burst in the precence of a non-ionic detergent, pH of the supernatant is adjusted to 6. Liquid or solid polyethylene glycol is added until supernatant is clarified.

Ultrafiltration

1.1 Outline of the production process
_[0053] Hepatitis B surface antigen may be produced by fermentation of an appropriate strain of Saccharomyces cerevisiae, for example that described in Harford et. al. (loc. cit.).
_[0054] At the end of large-_scale fermentation of the recombinant yeast strain, the cells are harvested and broken open in the presence of a mild surfactant such as Tween 20. The surface antigen is then isolated by a multistep extraction and purification procedure exactly as described above in Example 1 up to the step of the first gel permeation on Sepharose 4B.
The preparation of hepatitis B surface antigen is well documented. See for example, Harford et. al. in Develop. Biol. Standard 54, page 125 (1983), Gregg et. al. in Biotechnology, 5, page 479 (1987), EP-_A- 0 226 846, EP-_A-_0 299108 and references therein.

3.4.2 Required Devices for Downstream Processing

3.4.2.1 *Thiomersal free production process*

Centrifuge

Bead Mill (Grinding)

Ultrafiltration

4B Gel permeation chromatography

Anion exchange chromatography

Ultracentrifugation

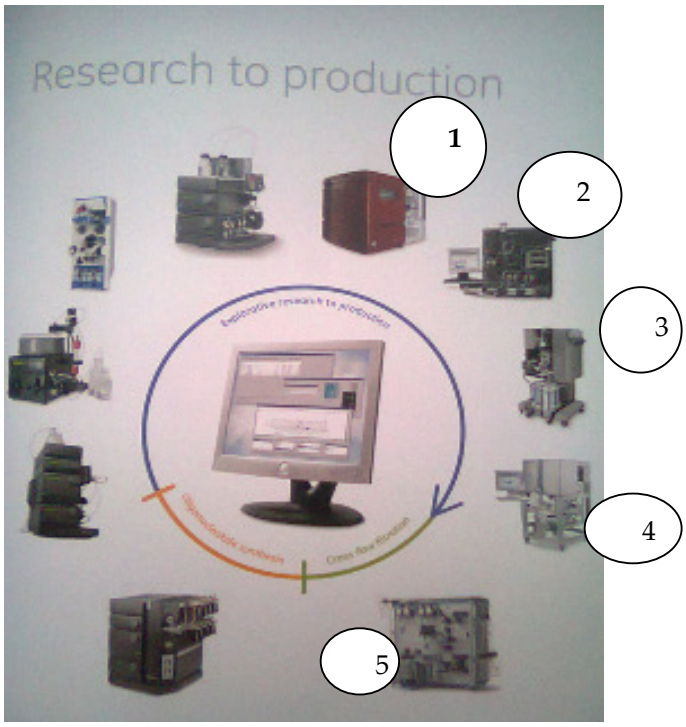
Desalting gel permeation chromatography

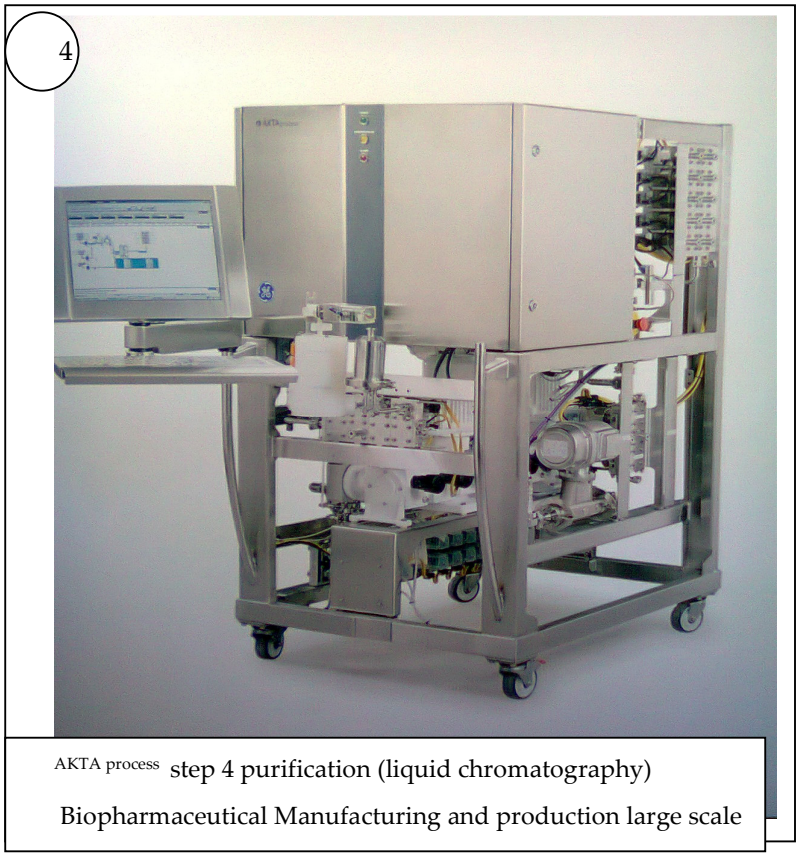
Sterile filtration

Requirement	Required for Step No.	Details	Details	Details
Device	4	Separation techniques	Designed to eliminate different types of yeast	
Material	4	aluminum hydroxide	adsorption	
Material	4 3	Tween 20	• Yeast strain • liberate the desired protein	Broken open
Material	4	thiomersal		
Material	4	Cysteine (amino acid)	Purification	
Material	5	potassium bichromate ($K_2Cr_2O_7$)		

Material	4	Sepharose 4B	extraction and purification	recombinant yeast strain (a gel permeation column)
Device	4	vapour generator		
Material	4	Mercury		
Device	2	Bead Mill (Grinding)		
Device	3	Ultrafiltration		
Device	3	4B Gel permeation chromatography		
Device	3	Anion exchange chromatography		
Device	3	Ultracentrifugation	Purification	a purified HBsAg
Device	4	Desalting gel permeation chromatography		
Device	4 1,3	Sterile filtration Centrifuge		
Material	3	CaCl2		
Material	4	the AUSZYME(kit)	Abbott Laboratories.	to assay HBsAg
Material	4	Cesium chloride CsCl	Ultracentrifugation	
Material	1	Glycerol glycerol and methanol	medium feeding medium feeding	First phase Second phase
Material	4	ELISA	immunological assay. Antibody response in mice 28 days.	
Material	3	DEAE-matrix	Ion exchange chromatography	For purification on Fast flow column.
Material	4	Sp-trisacryl ™ LS column	Supernatant of step 4 Is purified on it.	
Material	4	FORMALDEHYDE	Concentrated HBsAg eluate is treated with it	
Material	4	MINITAN ™ PELLICON ™	HBsAg solution concentrated on it.	
Material	4	SEPHACRYL 400	Purification of concentrated retentate	

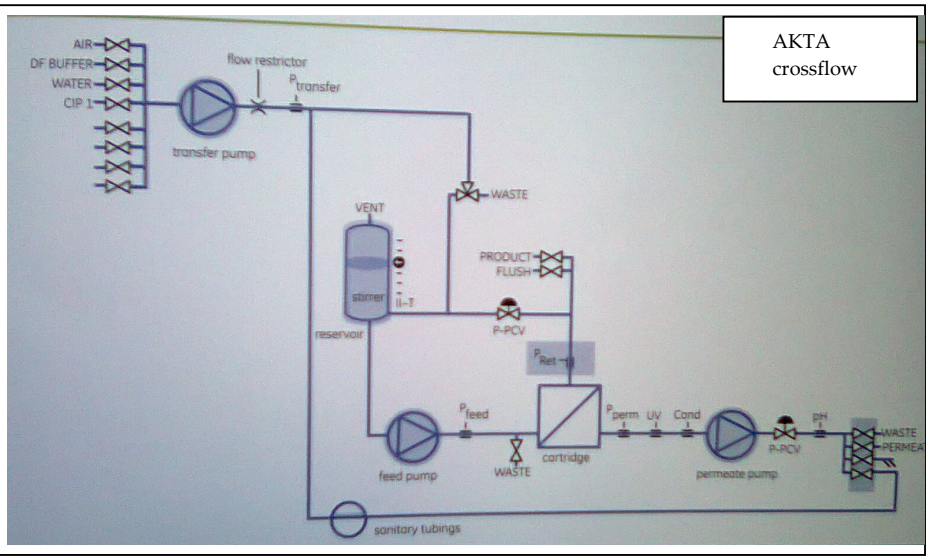
Material	4	0.2 MICRON FILTER	Eluate from SEPHACRYL 400	Filtration
Device	4	Ti 15 rotor from Beckman	Zonal Centrifugation	To eliminates residual lipids, DNA and minor protein contaminants from the HBsAg preparation. It is performed by it.
Material		DNase	Treatment of HBsAg	
Material		NaCl	dialysis	Buffer solution
Material		Na pO_4	dialysis	Buffer solution
Material		DTTor 2-mercaptoethanol		
Material		TRAVENOL (kit)	Solid phase radioimmunoassay for HBsAg	
Material		ABBOTT (kit)	Known standard	
Material		CHO (kit)	Recombinant HBsAg	
Material		HEPTAVAX B R (kit)	Plasma derived HBsAg vaccine	
Material		RECOMBIVAX HB (kit)	Yeast recombinant vaccine	
Material		AUSAB ETA (kit)	To test serum for presence of anti HBsAg antibodies	
Material		WHO (kit)	Reference serum for calibration	



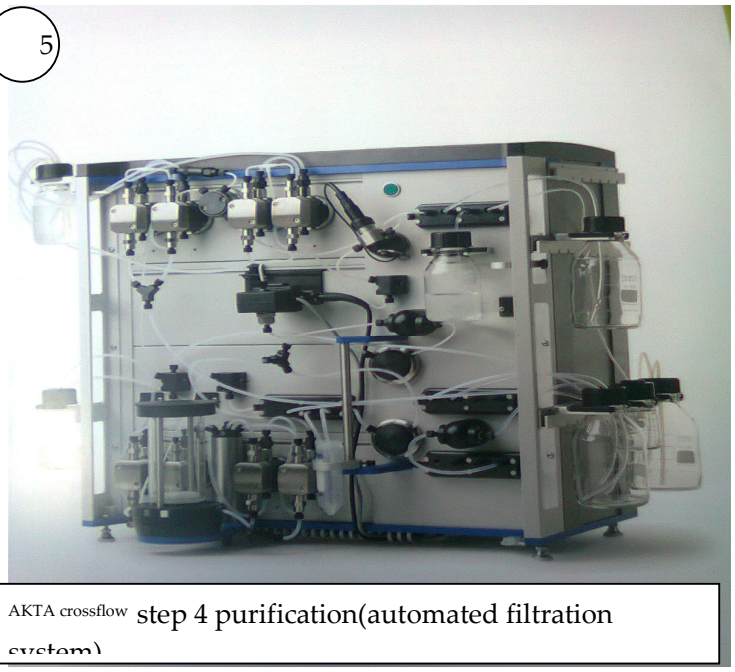


AKTA process step 4 purification (liquid chromatography)

Biopharmaceutical Manufacturing and production large scale



AKTA crossflow



AKTA crossflow step 4 purification(automated filtration system)

AKTA

AKTA

AKTApilo

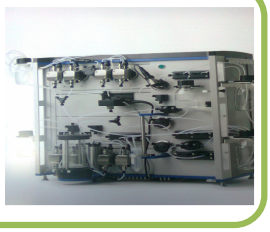
AKTA

GE Healthcare AKTA

Protein purification by design

AKTA

AKTA crossflow



3.4.2.2 Production Process of Hepatitis B Surface Antigen in the Presence of Thiomersal[3]

[0051]The Hepatitis B surface antigen (HBsAg) +of SB Biologicals hepatitis B monovalent vaccine (Engerix B®) is expressed as a recombinant protein in Saccharomyces cerevisiae (see Harford et al. loc. cit.). The 24 kD protein is produced intracellularly and accumulated in the recombinant yeast cells. At the end of the fermentation the yeast cells are harvested and disrupted in the presence of a mild surfactant such as Tween 20 to liberate the desired protein. Subsequently the cell homogenate, containing the soluble surface antigen particles, is prepurified in a series of precipitations and then concentrated via ultrafiltration.

[0052]Further purification of the recombinant antigen is performed in subsequent chromatographic separations. In a first step the crude antigen concentrate is subjected to gel permeation chromatography on Sepharose 4B medium. Thiomersal is present in the elution buffer at the 4B gel permeation chromatography step. The elution buffer has the following composition: 10 mM Tris, 5% ethylene glycol, pH 7.0, 50 mg/L thiomersal. Thiomersal is included in this buffer to control bioburden. Most of this thiomersal is removed during the subsequent purification steps including ion exchange chromatography, ultracentrifugation and desalting (gel permeation) so that purified bulk antigen preparations prepared by the original process contain about 1.2 μg and less than 2 μg of thiomersal per 20 μg of protein.

[0053] An Ion-Exchange chromatography step is performed using a DEAE-matrix and this pool is then subjected to a Cesium-gradient ultracentrifugation on 4 pre-established layers of different Cesium chloride concentrations. The antigen particles are separated from contaminating cell constituents according to their density in the gradient and eluted at the end of the centrifugation process. Cesium chloride is then removed from this pool by a second gel permeation on Sepharose gel.

[0054]When HBsAg is prepared by the process containing thiomersal in the 4B gel permeation buffer, protein concentrations of over 30 mg/ml are recovered in the pooled HBsAg containing fractions from the CsCl gradient, corresponding to an equivalent concentration of HBsAg as assayed by the AUSZYME kit from Abbott Laboratories.

[0055]The CsCl ultracentrifugation step usefully eliminates residual lipids, DNA and minor protein contaminants from the HBsAg preparation. It is performed by zonal centrifugation in a Ti 15 rotor from Beckman Instruments, Fullerton, Calif. at a speed of 30,000 rpm for about 40 to 60 hours. The sample to be purified is applied to layers of CsCl solution with final concentrations of 0.75, 1.5, 2.5 and 3.25 M CsCl. At the end of centrifugation the gradient is eluted into fractions. Fractions containing HBsAg may be identified by UV absorbance at 280 nm or by testing dilutions of the fractions with the AUSZYME kit. The HBsAg band is at a density of 1.17 to 1.23 g/cm^3.

[0056]The solution containing the purified HBsAg is sterile filtered before being used to make a vaccine formulation.

[0057]Purification from the yeast cell lysate is complex as the antigen is produced intracellularly and a series of separation techniques designed to eliminate different types of (yeast) contaminants are necessary to obtain pure bulk antigen. The steps of purification are important, as the product to be purified is a lipoprotein particle containing multiple copies of the surface antigen polypeptide and this structure must be maintained throughout the purification process. It is a particularity of this process that it yields surface antigen particles which are fully immunogenic without the need for further chemical treatment to enhance immunogenicity (compare EP0135435).

[3] From [Patent Engerix B]

[0058]The details of the production process are further described in European Patent 0199698.

3.4.3 Production and Characterization of Yeast-Derived HBsAg by a Thiomersal Free Process[4]

Production and Purification of Yeast-Derived HBsAg

1.1 Outline of the Production Process

[0059]Hepatitis B surface antigen may be produced by fermentation of an appropriate strain of Saccharomyces cerevisiae, for example that described in Harford et. al. (loc. cit.).

[0060]At the end of large-scale fermentation of the recombinant yeast strain, the cells are harvested and broken open in the presence of a mild surfactant such as Tween 20. The surface antigen is then isolated by a multistep extraction and purification procedure exactly as described above in Example 1 up to the step of the first gel permeation on Sepharose 4B.

1.2 Thiomersal-Free Purification Process

[0061]In the thiomersal free process the following two changes have been introduced compared to the process described in Example 1.

[0062]1. The elution buffer at the 4B gel permeation chromatography step no longer contains thiomersal.

[0063]2. Cysteine (2 mM final concentration) is added to the eluate pool from the anion exchange chromatography step.

[0064]It was found that omission of thiomersal from the 4B gel permeation buffer may result in precipitation of the HBsAg particles during the CsCl density gradient centrifugation step with loss of product and aggregation or clumping of the recovered antigen.

[0065]Addition of cysteine at 2 mM final concentration to the eluate pool from the preceding anion exchange chromatography step prevents precipitation and loss of antigen during CsCl density centrifugation.

[0066] 2. Cysteine is a preferred substance for this treatment as it is a naturally occurring amino acid and can be removed at the subsequent desalting step on a gel permeation column using Sepharose 4BCLFF as the column matrix.

[0067] There are no other changes in the manufacturing process compared to the process described in Example 1.

[0068] The thiomersal free process yields bulk antigen of a purity and with properties comparable to antigen from the process of Example 1.

1.2a

[0069] The thiomersal added to the 4B buffer at 50 μg/ml is thought to decompose and the resulting ethyl mercury may attach covalently to free sulphydryl groups on cysteine residues of the protein. The protein contains 14 cysteine residues of which 7 are located between positions 101 and 150.

[0070]This region of the protein is believed to be located at the surface of the particle and contain the major antigenic region of HBsAg including the immunodominant a region and the recognition site for the RF1 monoclonal antibody (Waters J et al, Postgrad. Med. J., 1987:63 (Suppl. 2): 51-56. and Ashton-Rickardt and Murray J. Med. Virology, 1989:29:196). Antigen purified with thiomersal

[4] From [Patent Engerix B]

present in the 4B gel permeation buffer contains about 0.5-0.6 μg mercury at the end of the purification process. This mercury is not fully removed by simple dialysis.

[0071]In one experiment, 0.56 μg Mercury per 20 μg protein was measured on bulk antigen preparation. This preparation was dialysed for 16 hours at room temperature against 150 mM NaCl, 10 mM $NaPO_4$ buffer pH 6.9. At the end of dialysis, a concentration of 0.33 μg Hg per 20 μg protein was measured.

[0072] In contrast, dialysis in the presence of a reducing agent such as L-cysteine at 0.1 to 5.0 mg/ml, DTT at 50 mM or 2-mercaptoethanol at 0.5 M, followed by a second dialysis to remove the reducing agent, results in reduction of the mercury content of the antigen preparation to less than 0.025 μg Mercury per 20 μg protein. This is the lowest limit of detection of the method.

[0073] The mercury content was determined by absorption spectrophotometry. The antigen is diluted in a solution containing 0.01% w/v of potassium bichromate ($K_2Cr_2O_7$) and 5% v/v of nitric acid. Standard solutions are prepared with thiomersal as the mercury source. The atomic absorption of sample and standard solutions is measured after vaporisation in a vapour generator, with a mercury-specific cathode at 253.7 nm. Atomic absorption of the dilution liquid is measured as blank. The mercury content of the sample is calculated via the calibration curves obtained from the standard solutions. Results are expressed as μg of mercury per 20 μg of protein.

3.5 Production of HBSAg in Mammalian Cells

Patent from 1991

US005242812A

United States Patent [19]

Even-Chen

[11] **Patent Number: 5,242,812**

[45] **Date of Patent: Sep. 7, 1993**

[54] **METHOD FOR PRODUCTION AND PURIFICATION OF HEPATITIS B VACCINE**

[75] Inventor: **Zeev Even-Chen**, Yavneh, Israel

[73] Assignee: **Bio-Technology General Corp.**, New York, N.Y.

[21] Appl. No.: **790,485**

[22] Filed: **Nov. 12, 1991**

Related U.S. Application Data

[63] Continuation of Ser. No. 480,166, Feb. 14, 1990, abandoned, which is a continuation-in-part of Ser. No. 307,777, Feb. 7, 1989, abandoned.

[51] **Int. Cl.**[5] **C07K 3/26**; C07K 3/28; C07K 3/20; C07K 15/14

[52] **U.S. Cl.** **435/70.3**; 424/88; 424/89; 435/69.3; 530/395; 530/412; 530/414; 530/415; 530/416; 530/417; 530/806; 935/65

[58] **Field of Search** 424/88, 89; 435/70.3, 435/69.3; 530/412, 414, 416, 417, 415, 806; 935/65

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,349,539	9/1982	Wampler	424/89
4,558,011	12/1985	Brzosko et al.	435/272
4,738,926	4/1988	Hamada et al.	435/239

OTHER PUBLICATIONS

Lee et al. 1987, Journal of the Chinese Biochemical Society 16(1): 7–14.

Molnar–Kimber et al. 1988, J. Virology 62(2): 407–416.

Zwerner et al. 1979, Methods in Enzymology, vol. 58: 221–229.

Primary Examiner—Keith C. Furman

Attorney, Agent, or Firm—John P. White

[57] **ABSTRACT**

Processes are provided for producing purified, hepatitis B surface antigen particles in mammalian cells which comprise culturing mammalian cells which produce the particles in a culture medium supplemented with a serum free of high molecular weight contaminant proteins and recovering the purified, hepatitis B surface antigen particles.

Removal of molecules having a molecular weight greater than about 3×10^5 daltons by prefractionation, for example, allows cells to be grown in culture media containing high levels of fetal calf serum, removes high molecular weight contaminant proteins which may be inhibitory to cell growth and simplifies purification of HBsAg since high molecular weight contaminant proteins are the major contaminants removed by purification processes.

43 Claims, 10 Drawing Sheets

PRODUCTION AND PURIFICATION SCHEME OF HBsAG PARTICLES

STEP 1 PRODUCTION OF HBsAG PARTICLES IN TISSUE CULTURE SYSTEM USING FCS - SUPPLEMENTED CULTURE MEDIA THAT WAS PREFRACTIONATED ON A PELLICON™ 300,000 MW CUT OFF MEMBRANE - ONLY FILTRATE (PROTEINS< 300,000 MW) IS ADDED TO CELL CULTURE. RETENTATE ON THE MEMBRANE (PROTEINS> 300,000 MW) IS DISCARDED.

↓

STEP 2 COLLECT CULTURE MEDIA DAILY, POOL AND PURIFY BY FIRST CLARIFYING CRUDE MEDIUM CONTAINING HBsAG PARTICLES ON A PELLICON™ 0.22 MICRON MEMBRANE - ONLY FILTRATE IS PURIFIED FURTHER. RETENTATE (CELLS AND CELL DEBRIS) ON MEMBRANE IS DISCARDED.

↓

STEP 3 CONCENTRATION OF HBsAG PARTICLES FROM CLARIFIED CRUDE MEDIUM (FILTRATE OF STEP 2) AND DIALYSIS AGAINST PBS ON A PELLICON™ 300,000 MW CUT OFF MEMBRANE - ONLY RETENTATE IS PURIFIED FURTHER (PROTEINS ABOVE 300,000 MW). FILTRATE IS DISCARDED (PROTEINS BELOW 300,000 MW). RETENTATE CONCENTRATED FURTHER ON MINITAN™ 300,000 CUT-OFF MEMBRANE.

↓

STEP 4 RETENTATE OF STEP 3 IS PURIFIED BY GEL FILTRATION I ON A SEPHACRYL S-400™ COLUMN. FOLLOWED BY CONCENTRATION OF ELUTED HBsAG PEAK FRACTIONS ON MINITAN™ (300,000 MW CUT OFF MEMBRANE).

↓

STEP 5 CONCENTRATED HBsAG ELUATE OF STEP 4 PURIFIED FURTHER BY GEL FILTRATION II ON SEPHACRYL S-400™ COLUMN, FOLLOWED BY CONCENTRATION OF ELUTED HBsAG PEAK FRACTIONS ON MINITAN™ (300,000 MW CUT OFF MEMBRANE).

Figure 1

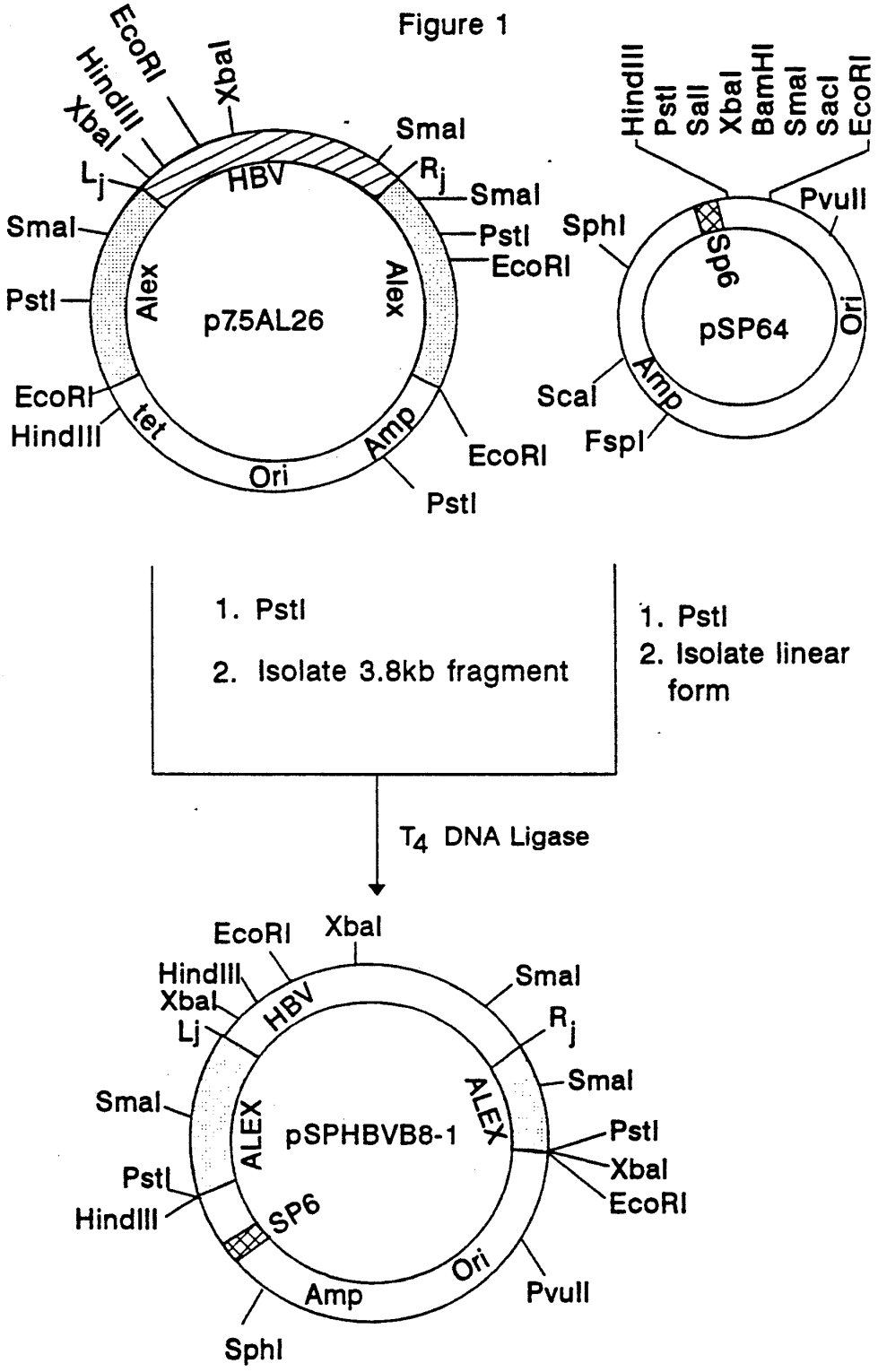

EcoRI
HindIII
Xbal
Xbal
Lj
HBV
SmaI
Rj
SmaI
PstI
EcoRI
SmaI
PstI
Alex
p7.5AL26
Alex
EcoRI
HindIII
tet
Ori
Amp
EcoRI
PstI
HindIII
PstI
SalI
Xbal
BamHI
SmaI
SacI
EcoRI
PvuII
SphI
SP6
pSP64
Ori
ScaI
Amp
FspI
1. PstI
2. Isolate 3.8kb fragment
1. PstI
2. Isolate linear form
T4 DNA Ligase
EcoRI
Xbal
HindIII
Xbal
HBV
SmaI
Lj
Rj
SmaI
SmaI
ALEX
pSPHBVB8-1
ALEX
PstI
Xbal
EcoRI
PstI
HindIII
SP6
Amp
Ori
PvuII
SphI

Figure 4

PRODUCTION AND PURIFICATION SCHEME OF HBsAG PARTICLES

STEP 1 PRODUCTION OF HBsAG PARTICLES IN TISSUE CULTURE SYSTEM USING FCS - SUPPLEMENTED CULTURE MEDIA THAT WAS PREFRACTIONATED ON A PELLICON™ 300,000 MW CUT OFF MEMBRANE - ONLY FILTRATE (PROTEINS< 300,000 MW) IS ADDED TO CELL CULTURE. RETENTATE ON THE MEMBRANE (PROTEINS> 300,000 MW) IS DISCARDED.

↓

STEP 2 COLLECT CULTURE MEDIA DAILY, POOL AND PURIFY BY FIRST CLARIFYING CRUDE MEDIUM CONTAINING HBsAG PARTICLES ON A PELLICON™ 0.22 MICRON MEMBRANE - ONLY FILTRATE IS PURIFIED FURTHER. RETENTATE (CELLS AND CELL DEBRIS) ON MEMBRANE IS DISCARDED.

↓

STEP 3 CONCENTRATION OF HBsAG PARTICLES FROM CLARIFIED CRUDE MEDIUM (FILTRATE OF STEP 2) AND DIALYSIS AGAINST PBS ON A PELLICON™ 300,000 MW CUT OFF MEMBRANE - ONLY RETENTATE IS PURIFIED FURTHER (PROTEINS ABOVE 300,000 MW). FILTRATE IS DISCARDED (PROTEINS BELOW 300,000 MW). RETENTATE CONCENTRATED FURTHER ON MINITAN™ 300,000 CUT-OFF MEMBRANE.

↓

STEP 4 RETENTATE OF STEP 3 IS PURIFIED BY GEL FILTRATION I ON A SEPHACRYL S-400™ COLUMN. FOLLOWED BY CONCENTRATION OF ELUTED HBsAG PEAK FRACTIONS ON MINITAN™ (300,000 MW CUT OFF MEMBRANE).

↓

STEP 5 CONCENTRATED HBsAG ELUATE OF STEP 4 PURIFIED FURTHER BY GEL FILTRATION II ON SEPHACRYL S-400™ COLUMN, FOLLOWED BY CONCENTRATION OF ELUTED HBsAG PEAK FRACTIONS ON MINITAN™ (300,000 MW CUT OFF MEMBRANE).

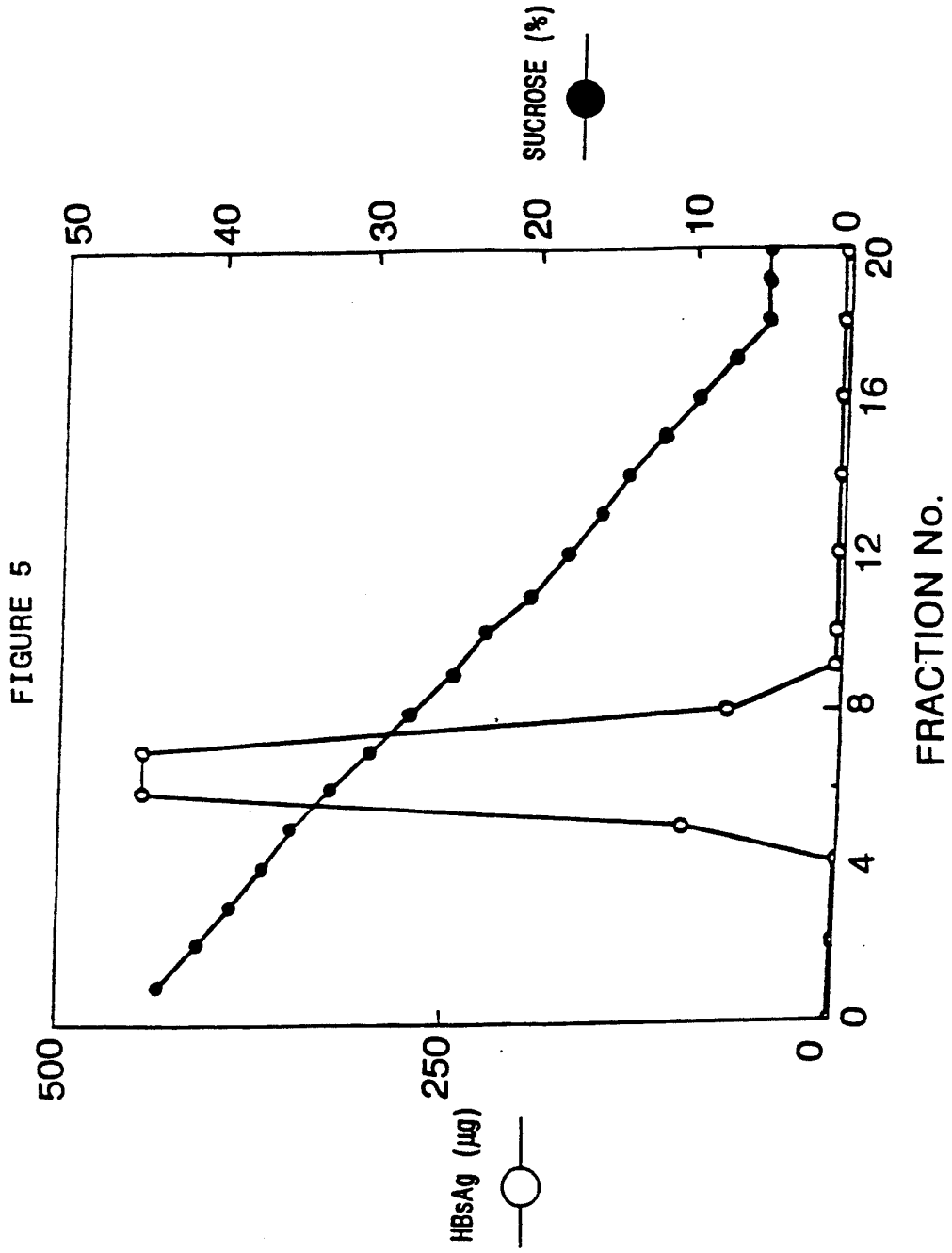

SUCROSE (%)
50
40
30
20
10
0
500
250
0
HBsAg (μg)
0
4
8
12
16
20
FRACTION No.

FIGURE 5

FIGURE 6B

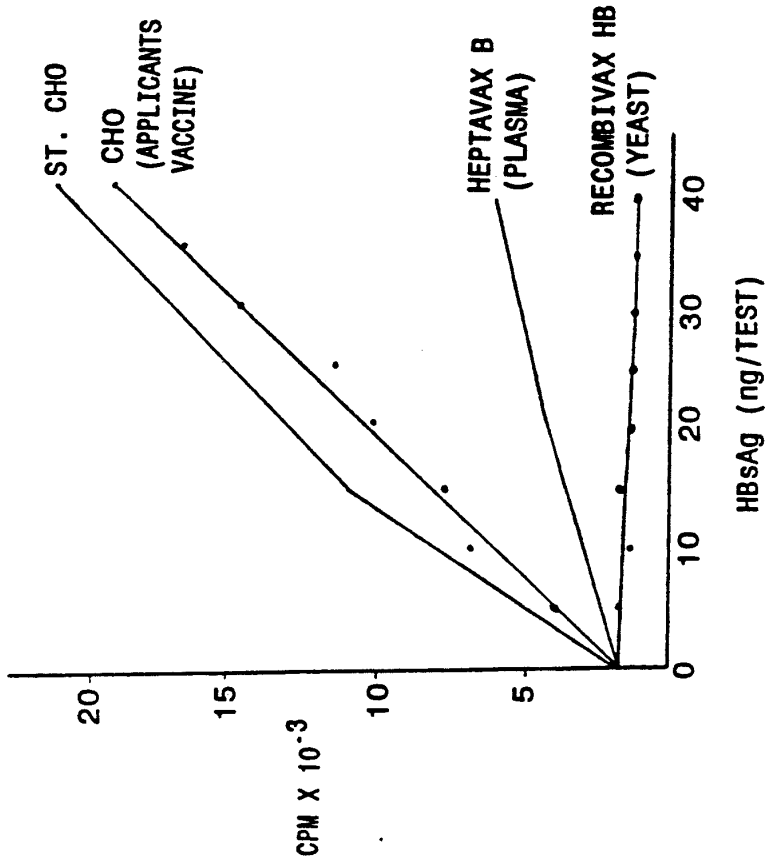


FIGURE 6A

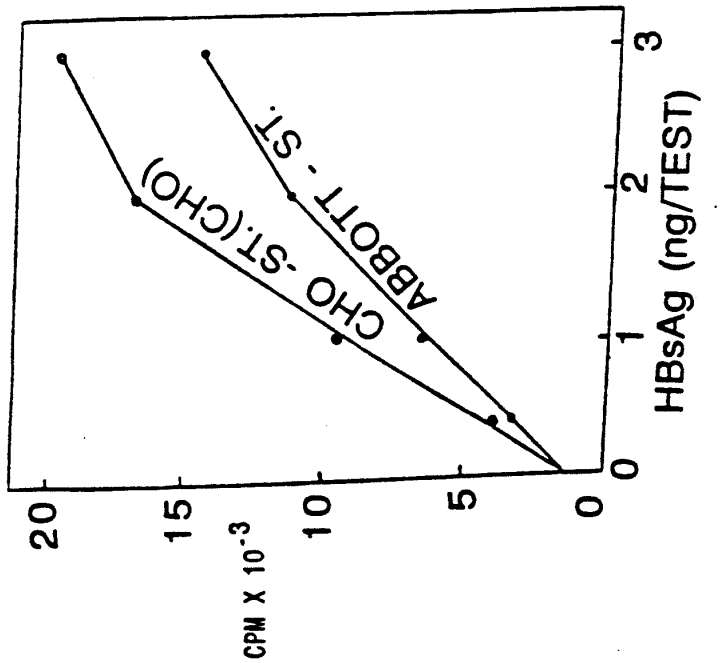
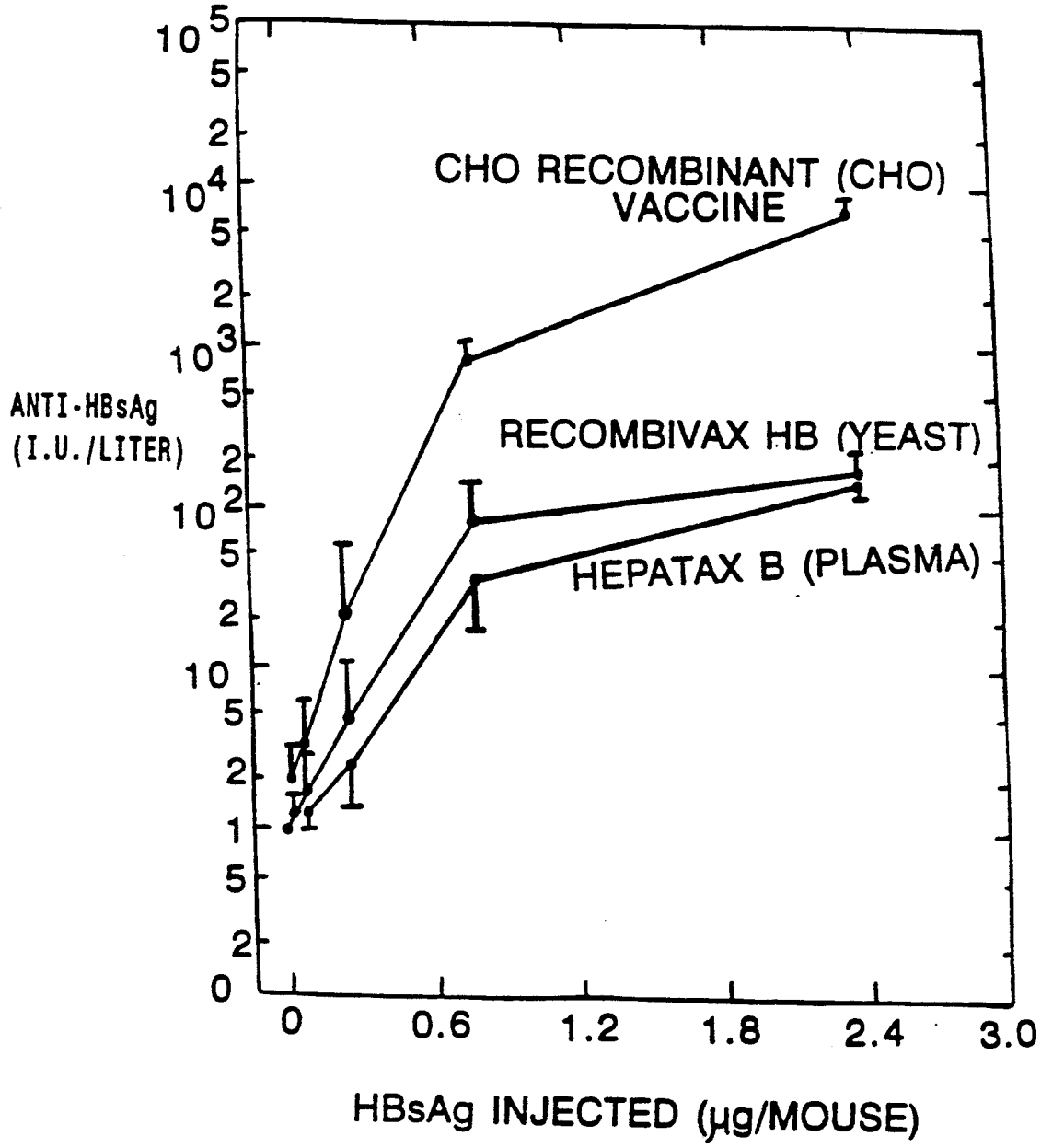


FIGURE 7


ANTI-HBsAg
(I.U./LITER)
CHO RECOMBINANT (CHO)
VACCINE
RECOMBIVAX HB (YEAST)
HEPATAX B (PLASMA)
0
0.6
1.2
1.8
2.4
3.0
HBsAg INJECTED (µg/MOUSE)

3.6 Overview: Required Devices for Engerix B Downstream Processing

I-centrifugation

II-grinding bead mill

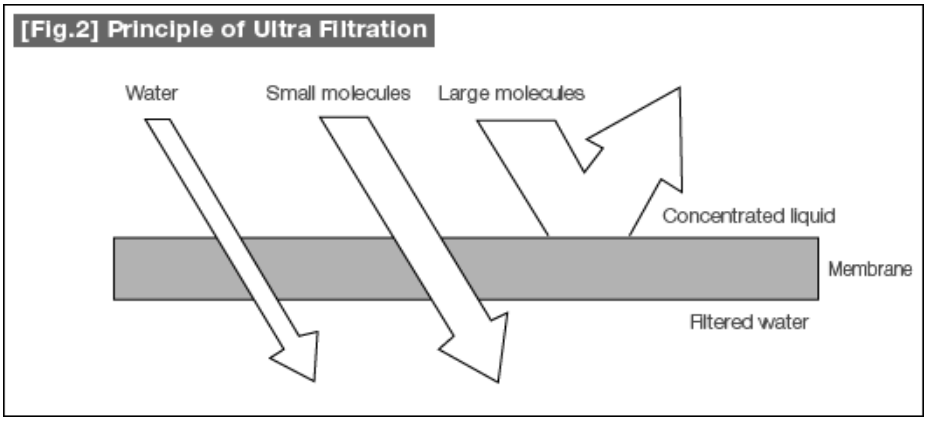
Link: http://www.omni-inc.com/omni-bead-ruptor-12-homogenizer-p-522.html

III- centrifugation

IV-centrifugation

V- ultrafiltration

link: http://www.solarisgroup.org/tytan-micro-ultra-filtration.html



VI- 4b gel permeation chromatography

chromatographic gel permeation column : A typical Waters GPC instrument including A. sample holder, B.Column C.Pump D. Refractive Index Detector E. UV-vis Detector

VII- anion exchange chromatography

chromatographic anion exchange column

VIII-ultracentifugation

link:http://www.medicalexpo.com/prod/beckman-coulter-international-sa/high-performance-bench-top-laboratory-centrifuges-75322-507590.html

IX-desalting gel permeation

desalting gel permeation

X-steril filtration

الالات اللازمة لعملية ال Downstream :

السعر	الشركة	المرحلة	الالة
...	...	I , III , IV	centifuge
...	...	V	ultrafiltration
...	...	VI	Chromatographic gel permeation columns
...	...	VII	Chromatographic anion exchange columns
...	...	VIII	ultracentrifuge
...	...	IX	Desalting gel permeation

3.7 Chromatographic Purification device – process scale

3.7.1 Purification system:

From "ÄKTAprocess™ Operating Instructions": Chapter 5 (Operation)

3.7.2 ÄKTAprocess

System with standard configuration: front view

The illustration below shows a front view of the standard configuration of ÄKTAprocess

Part	Function
1	pH probe
2	Battery limit: System outlets (2)
3	pH probe calibration holder
4	**EMERGENCY STOP**
5	Battery limit: Column 1 connections (2)
6	Flow meter
7	Battery limit: Common waste outlet
8	Swiveling wheel with brake (4)
9	Common waste collection cup
10	Pre-column pressure meter
11	Skid maneuvering handle (2)
12	Air trap
13	Operator console with keyboard and monitor
14	Indicator lamp - **ALARM**
15	Indicator lamp - **RUN/PAUSE**
16	Indicator lamp - **POWER**

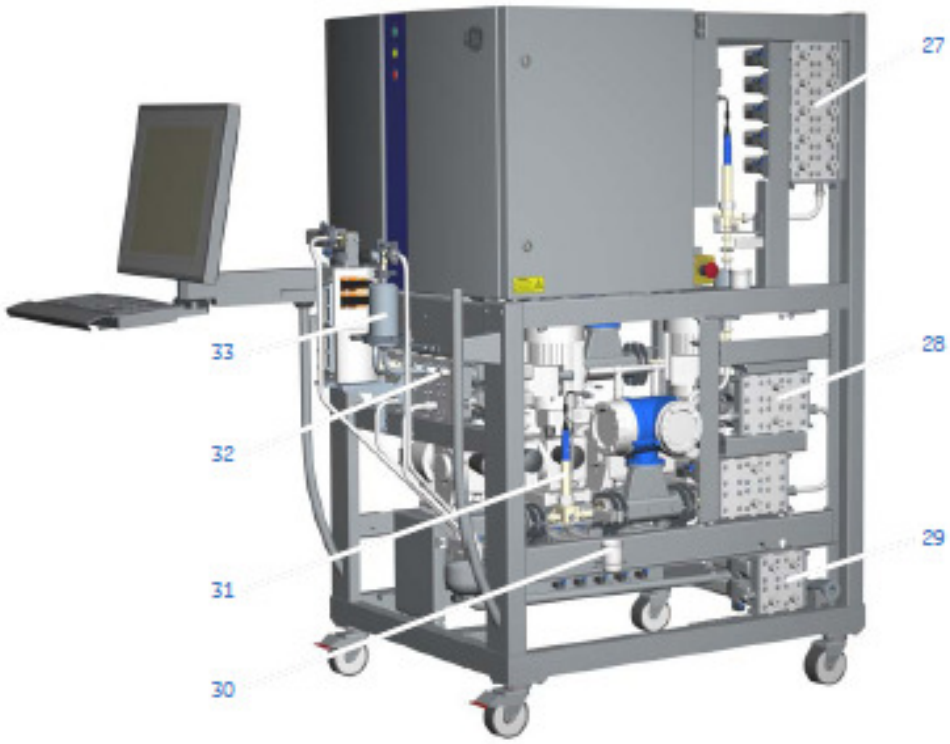
rear view

The illustration below shows a rear view of the standard configuration of ÄKTAprocess.

Part	Function
17	Pneumatic air supply connection port
18	**SYSTEM POWER SWITCH**
19	**EMERGENCY STOP**
20	Pressure meter
21	Pre-column conductivity meter
22	System pump A
23	Moveable air sensor
24	Battery limit: System inlets (2)
25	Post-column conductivity meter
26	System label

System with all options: front view

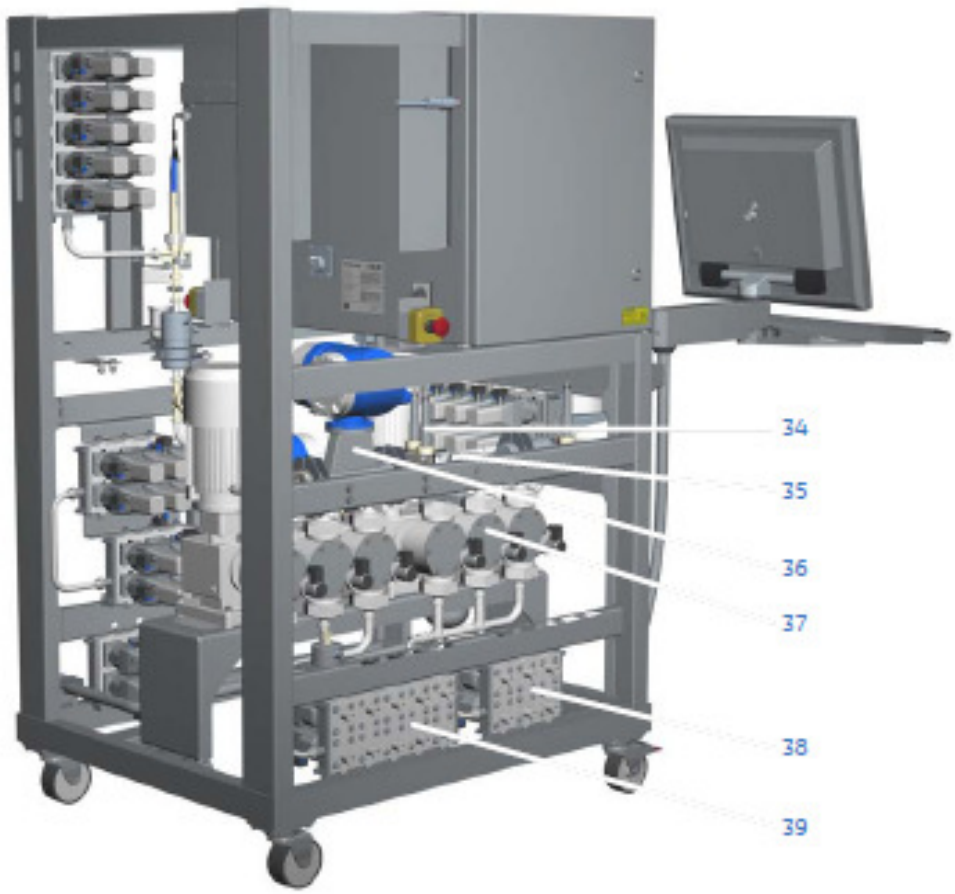
The illustration below shows a front view of an example of ÄKTAprocess with all optional Components



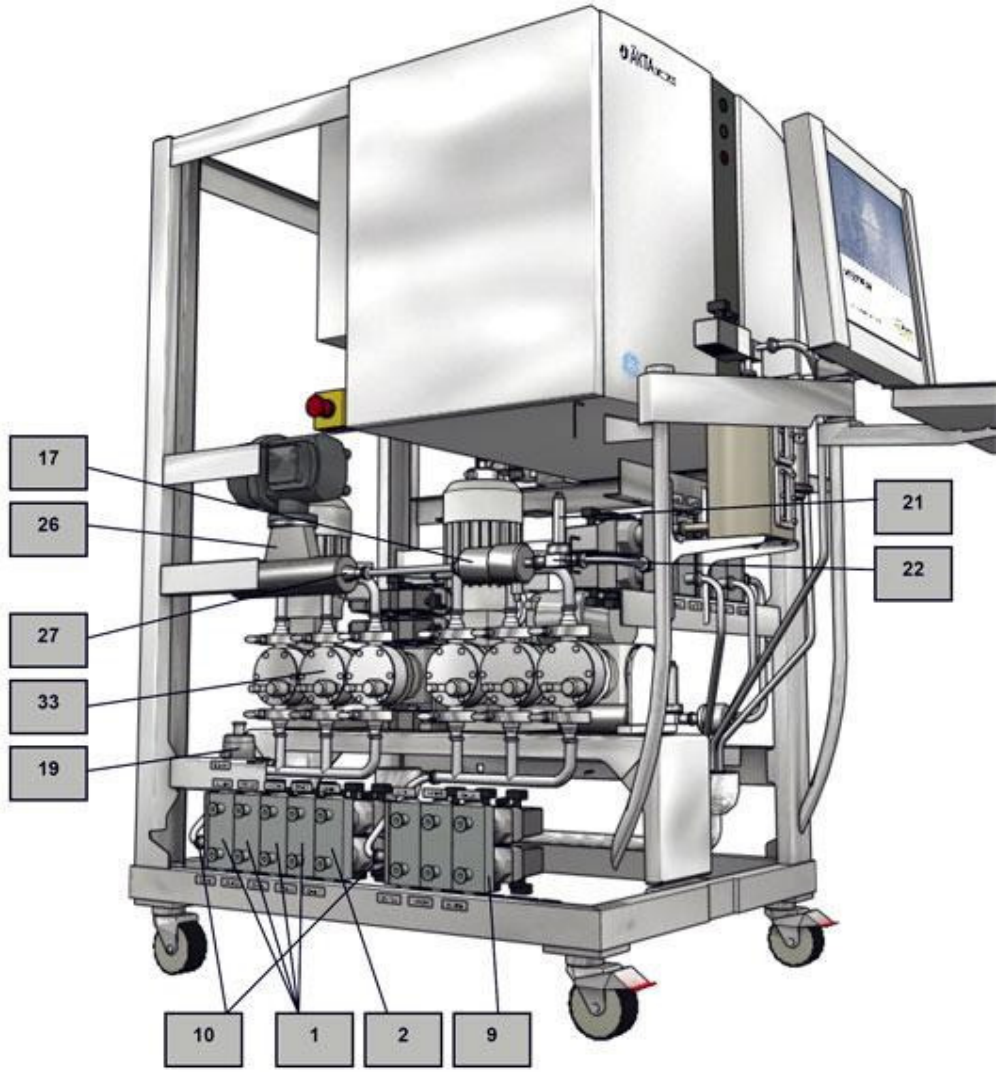
Part	Function
27	Battery limit: System outlets (10)
28	Battery limit: Column 2 connections (2)
29	Battery limit: CIP / AxiChrom™ valves
30	Pre-column pH probe calibration cup
31	Pre-column pH probe
32	Sample pump inlet
33	In-line filter

rear view

The illustration below shows a rear view of an example of ÄKTAprocess with all optional Components

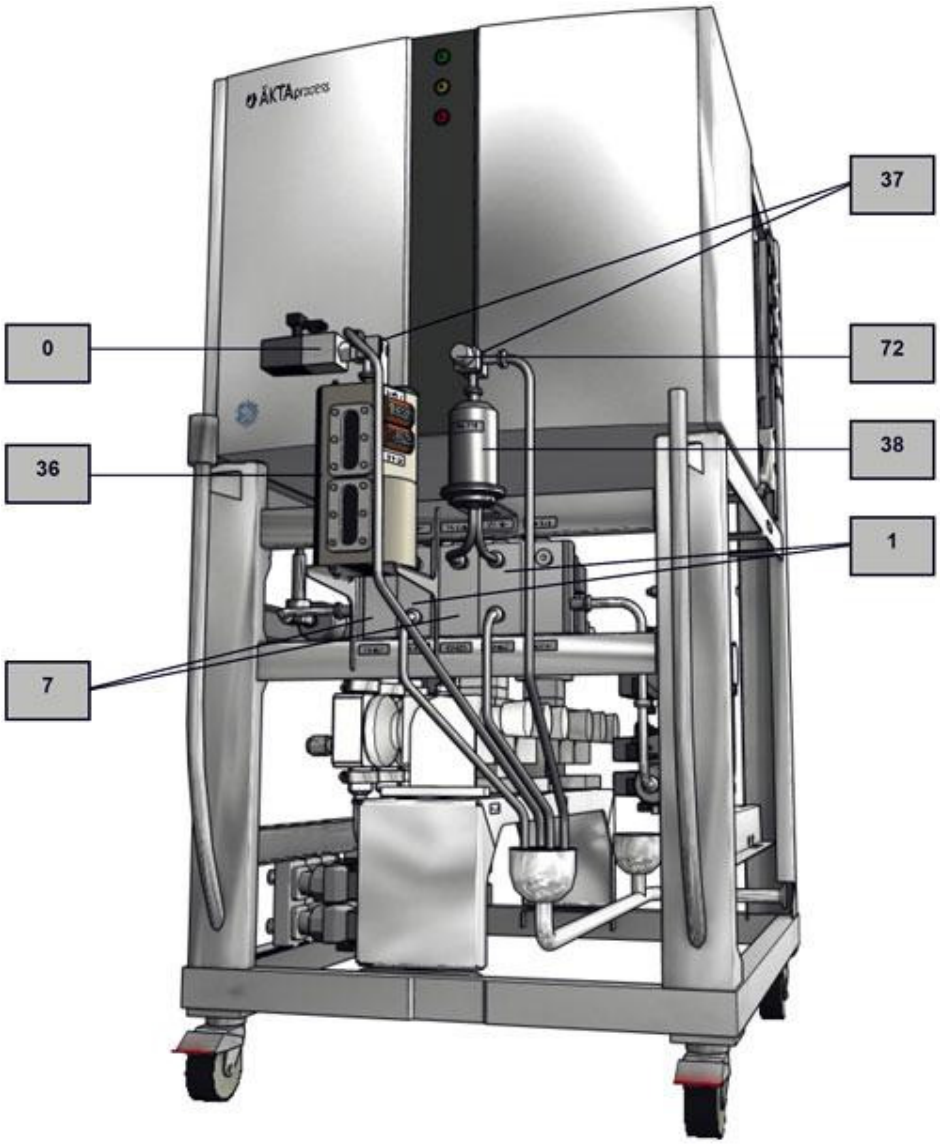


Part	Function
34	Pressure meter with PCV option (2)
35	Pressure control valve
36	Flow meter
37	System pump B
38	Battery limit: Buffer B inlets (6)
39	Battery limit: Buffer A inlets (10)



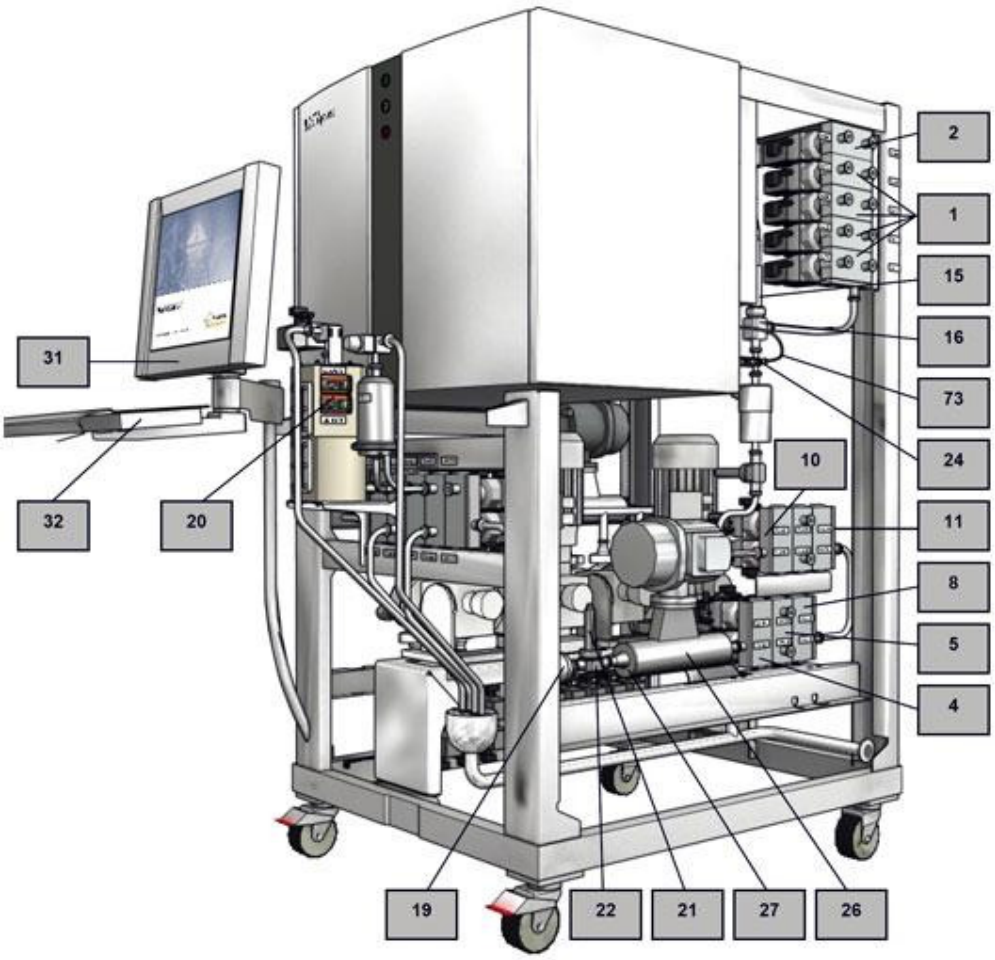
1	Valve body centre, i.d. 10 mm
2	Valve body right, i.d. 10 mm
9	Valve block mounting plate DN08, DN10
10	End connection left, i.d. 10 mm
12	Block rod 1, to valve bodies DN08 & DN10
12	Block rod 2, to valve bodies DN08 & DN10
12	Block rod 3, to valve bodies DN08 & DN10
12	Block rod 4, to valve bodies DN08 & DN10
12	Block rod 5, to valve bodies DN08 & DN10

17	Cable conductivity sensor Dsub-ODU, length = 2.2 m
17	Assembly / Disassembly tool to conductivity sensor
17	O-ring kit
17	Conductivity sensor,i.d. 8 mm
19	Cable air sensor RJ12-ODU6, length = 5.4 m
19	Cable air sensor RJ12-ODU6, length = 2.4 m
19	Air sensor i.d. 10 mm
21	Cable to pressure sensor RJ12-Binder
21	Cable to pressure sensor RJ12-Binder
21	Pressure sensor PAA35LXH 12bar
22	Pressure flow cell,i.d. 10 mm
22	O-ring 17.2x1.78 mm
26	Local display for flow meter
26	Flow meter DN10
27	TC adaptor, i.d. 10 mm
27	O-ring kit 17.04x3.53 mm, for TC adaptor
33	Clamp Tool CV Lewa Pumps
33	Pump head hygenic forpump 90 PP EPDM
33	Valvebody & guide kit90 PP
33	Wear & Tear Kit
33	Pump Hygienic 3x90 PP EPDM



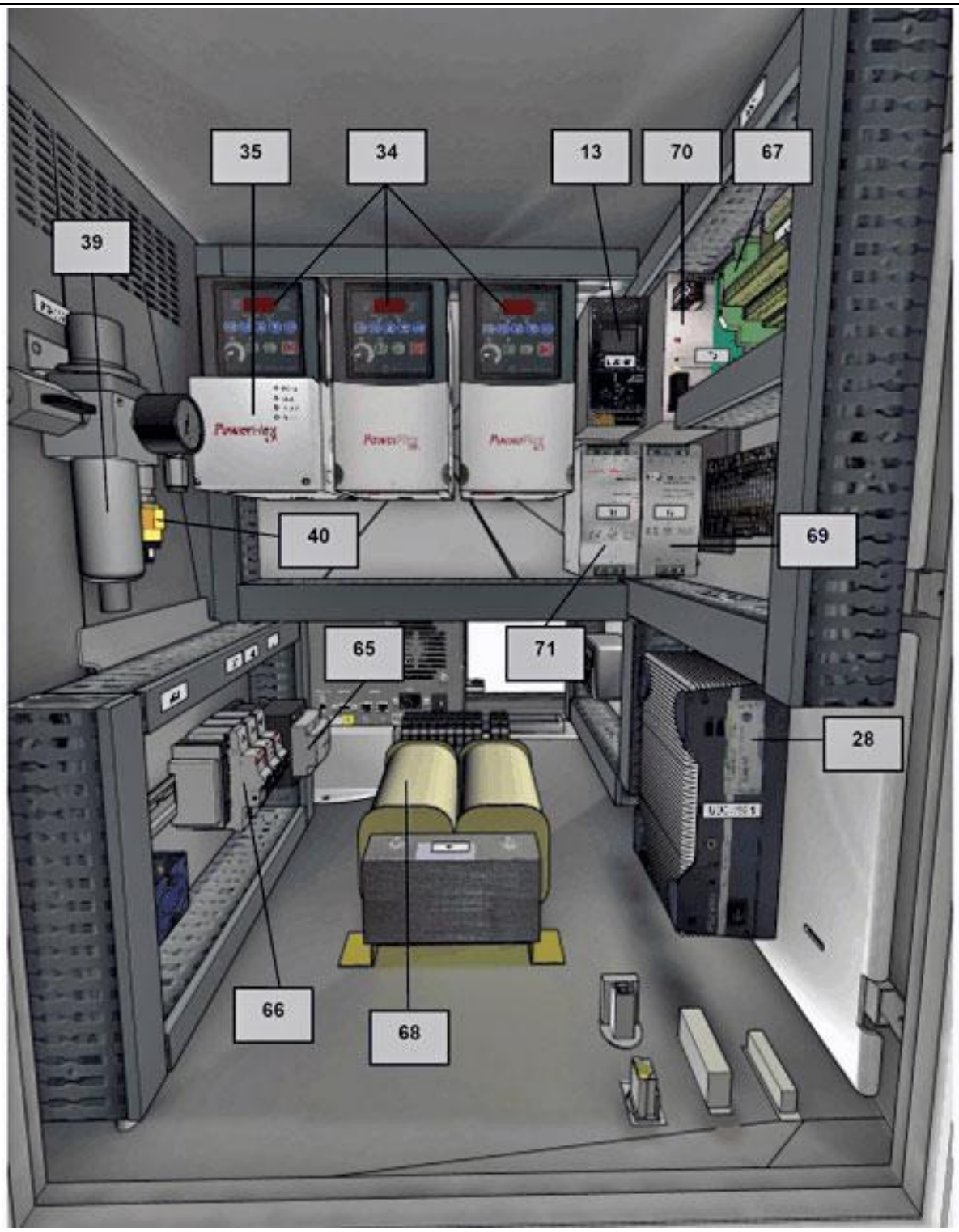
1	Valve body centre, i.d. 10 mm
7	Actuator DN10 90
7	Valve body air trap, i.d. 10 mm
12	Block rod 1, to valve bodies DN08 & DN10
12	Block rod 2, to valve bodies DN08 & DN10
12	Block rod 3, to valve bodies DN08 & DN10
12	Block rod 4, to valve bodies DN08 & DN10
12	Block rod 5, to valve bodies DN08 & DN10
36	O-ring kit for air trap 10 mm
36	Sight glass 34x95x17 mm
36	Air trap, i.d. 10 mm
37	Actuator DN08 0

37	Valve body angled right, i.d. 3 mm
37	Diaphragm DN08
38	Clamp, 3 inch TC
38	Gasket, 91 mm TC, i.d. 73 mm
38	Filter house, i.d. 10 mm
38	Filter Cartridge
72	Actuator manual valve DN08



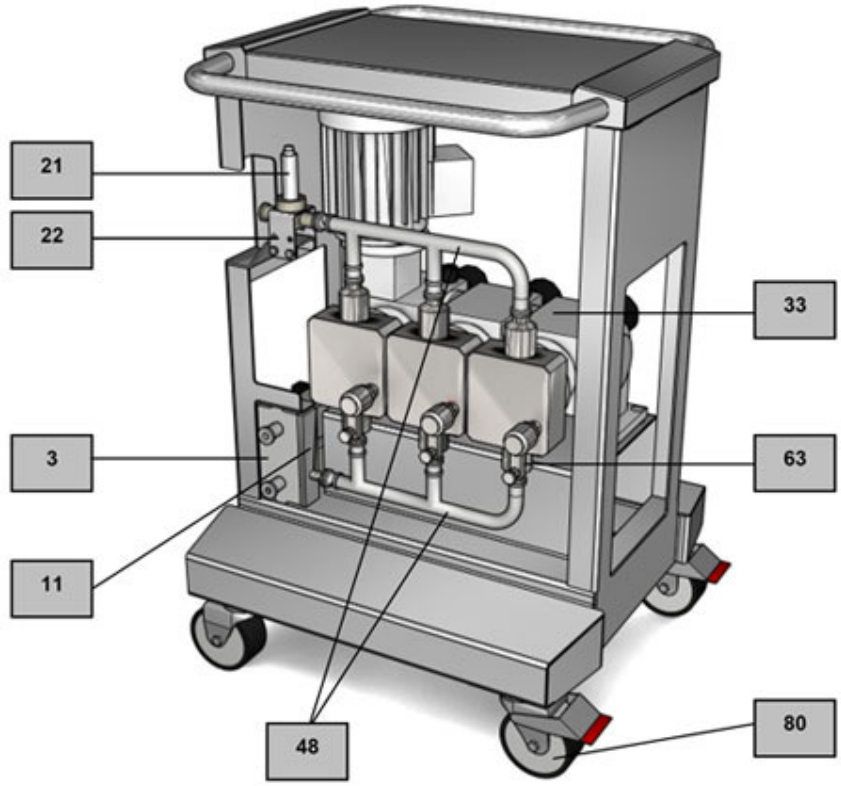
1	Valve body centre, i.d. 10 mm
2	Valve body right, i.d. 10 mm
4	Valve body right, i.d. 10 mm
5	Valve body centre, i.d. 10 mm
8	Valve body left, i.d. 10 mm
10	End connection left, i.d. 10 mm
11	End connection right, i.d. 10 mm

12	Block rod 1, to valve bodies DN08 & DN10
12	Block rod 2, to valve bodies DN08 & DN10
12	Block rod 3, to valve bodies DN08 & DN10
12	Block rod 4, to valve bodies DN08 & DN10
12	Block rod 5, to valve bodies DN08 & DN10
15	pH cable CPK9 TOP 68, length = 5 m
15	pH electrode
16	Adaptor pH flow cell
16	Adaptor nut
16	Cleaning-in-place (CIP) Cap to pH flow cell
16	pH flow cell
19	Cable air sensor RJ12-ODU6, length = 5.4 m
19	Cable air sensor RJ12-ODU6, length = 2.4 m
19	Air sensor i.d. 10 mm
20	Level sensor capasitive IFM
21	Cable to pressure sensor RJ12-Binder
21	Cable to pressure sensor RJ12-Binder
21	Pressure sensor PAA35LXH 12bar
22	Pressure flow cell,i.d. 10 mm
22	pH and pressure flowcell i.d. 10 mm
22	O-ring 17.2x1.78 mm
24	UV flow cell, i.d. 1 inch
24	UV flow cell, i.d. 8 mm
24	O-ring kit
26	Local display for flow meter
26	Flow meter DN10
27	TC adaptor, i.d. 10 mm
27	O-ring kit 17.04x3.53 mm, for TC adaptor
31	Console complete without touch screen
31	Console complete with touch screen
32	Keyboard UK/US IP65
73	Long optical fibre kit, 500 mm



13	Profibus/AS-I gateway
13	Wire ASI-cable 2-port buscable, length = 13 m
13	Connector ASI-cable
28	Computer AAEON AEC-6850

28	Communications cable RJ45-RJ45/IP67, length = 10 m
34	Frequency converter 0.75kW / 1ph
35	Frequecy converter profibus interface
39	Pressure regulator
40	Pressure switch PM11-NA
65	Contactor 25A 24V DC
66	Fuse 10 A, 1 POL
67	Opto Coupler DEK-OE-24DC
68	Trafo Mains 2000VA 1-PHASE
69	Power supply AC/DC 24V 75W DIN
70	Power supply ASi, 30 VDC 2.4 A
71	Power supply AD/DC 12V 75 W DIN



3	Actuator DN10 0
3	O-ring, DN10
3	Block rod 1, to valve bodies DN08 & DN10
3	Pneumatic quick fitting T M5 / i.d. 6 mm

3	Pneu. quick fitting (L)M5 / i.d. 6 mm
3	Diaphragm DN10
3	Valve body left, i.d. 10 mm
11	End connection right, i.d. 10 mm
21	Cable to pressure sensor RJ12-Binder
21	Cable to pressure sensor RJ12-Binder
21	Pressure sensor PAA35LXH 12bar
22	Pressure flow cell,i.d. 10 mm
22	O-ring 17.2x1.78 mm
33	Clamp Tool CV Lewa Pumps
33	Pump head hygenic forpump 90 PP EPDM
33	Valvebody & guide kit90 PP
33	Wear & Tear Kit
33	Pump Hygienic 3x90 PP EPDM
48	Pump manifold, i.d. 10 mm
63	TC clamp kit, 25 mm TC
63	O-ring, DN10
63	TC gasket 25 mm, i.d. 15 mm
63	TC Gasket, 25 mm, i.d. 16.5 mm
63	End cap, 25 mm TC
63	TC-gasket kit, i.d. 10 mm
80	Wheel kit

3.7.2.1 *Structural components*

Electric cabinet

The electric cabinet serves as the container for all electrical and pneumatic equipment.

Skid

The rigid stainless steel structure supports all process components and the electric cabinet.
The structure is designed for handling in a production environment and to be easy to move and keep clean.
The steel structure protects all installed components while still allowing easy access.
The structure occupies a small box-shaped space that makes it easy to fit into any location in the production facility.

Control system

ÄKTAprocess is fully automated by means of the UNICORN control system. Once the required methods are created and approved, a non-expert user can safely operate the system.

Control unit

A **CU-960** control unit is the controlling interface between UNICORN and the components of ÄKTAprocess.

The **CU-960** control unit is located inside the cabinet.

Computer

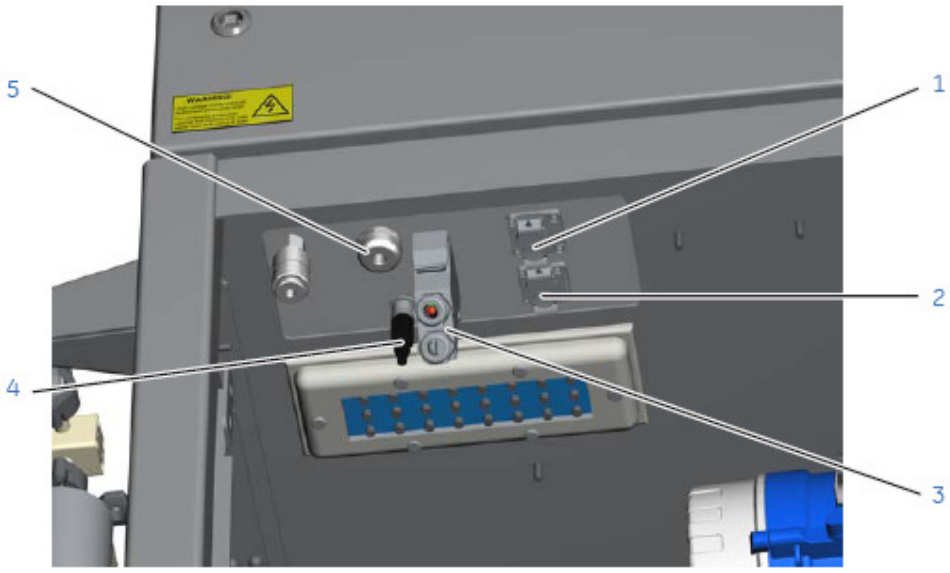
The computer is built into the cabinet and fully protected from the outside environment.

User console

The display and input equipment is ergonomically designed for usage in a clean production environment.

Communications

Communication with most controlled components mounted outside the cabinet uses the PROFIBUS™ industry standard communication protocol and hardware. The PROFIBUS connection and other communication ports are located on the underside of the electrical cabinet, as shown in the illustration below. For information on where to connect the PROFIBUS signal cable to the AxiChrom Master, see the *AxiChrom User Manual*



Part	Function
1	USB connection port
2	Ethernet connection port
3	Customer I/O connection
4	PROFIBUS connection
5	UPS Power

Inlets and outlets

This section describes the inlets and outlets, including the drain outlet, of ÄKTAprocess that are provided in the standard configuration.

Connections

The standard configuration of ÄKTAprocess has two inlets, two outlets and connections

for one column. As shown in the flowchart in *Section 3.5 Flowchart, on page 72* ÄKTAprocess has a moveable air sensor that may be connected to any inlet.
For standard ÄKTAprocess configurations the pressure on the inlets should be in the range 0 to 0.2 bar. The outlets can handle backpressure up to 1 bar.

Drains

All drains from ÄKTAprocess are collected to a single drain outlet. The drains are first collected in an open cup to ensure that no back pressure is applied on any parts of the processing system.

Meters and sensors

This section describes the meters and sensors that are installed as standard components of ÄKTAprocess.

Overview

ÄKTAprocess is provided with a set of sensors and meters that provide data to the control system, enabling it to control the progress and detect the performance of the process in a satisfactory way.
The basic system setup includes meters and sensors that measure pressure, flow, conductivity (Cond), pH, air, temperature and UV. Measurement of these parameters enables basic isocratic operation and the air sensor before the column also makes sure that no air enters the column during processing.

Flow meter measurement principle

The measuring principle of the flow meter is based on the controlled generation of Coriolis forces. Refer to the flow meter manual in the product documentation package for more information.

System pump

This section describes the basic ÄKTAprocess system pump. In the standard configuration, a single system pump is provided that supports isocratic operation.

Pump type

The system pump is a triple head diaphragm pump, or a 5-headed diaphragm pump for flow rates 45 to 2000 l/h. The process wetted parts of the pump heads are effectively sealed from non-sanitary components of the pump.
The pump is provided with stroke length adjustment knobs. These are factory preset at delivery and must not be adjusted by the user.

Pump stroke frequency

The pump stroke frequency is controlled by the flow that is set in the UNICORN control software.

Safety monitoring

The system is protected from exceeding the high pressure limit by the electronic module **ALP-900**, an air, level and pressure monitoring system that is situated inside the cabinet.
The **ALP-900** monitors:
1 The pressure in each pressure sensor
2 The pressure difference between each pressure sensor
3 The temperature in the process liquid
If any of the monitored parameters reaches a critical limit, the **ALP-900** will shut down the pumps independently from the UNICORN control system.

Air trap

An air trap is installed in the flow path of ÄKTAprocess. This section describes the air trap and the sensors that are used for liquid level control.

Air trap function

The function of the air trap is to de-gas buffers. A vortex is created in the air trap and the liquid in the air trap is pressed downwards and outwards by the centrifugal force generated while air is separated in the center of the chamber. The rotation eliminates pockets of stagnant liquid, which prevents unwanted build-up of solids (e.g., bacterial cells) and simplifies the cleaning of the air trap.

Level sensors

Two sensors for automatic liquid level control are installed in the air trap. This sensor assembly consists of a high and a low level sensor. The sensors must be re-calibrated if the LED indicator displays a red light while the liquid level is still far from the low or high level markers.
It is recommended that after a power down a calibration of the level sensors should always be performed. See Section 6.6.2 Air trap calibration, on page 153.
Note:
When the air trap is filled with liquid that foams easily, for example liquids containing detergents and protein solutions (sample), large volumes of air should not be allowed to enter into the air trap. If foam is formed it may interfere with the automatic liquid level control.
Note:
Always position a movable air sensor at the inlet of the sample or detergentcontaining liquid. The air sensor will set the system to **Pause** when air is detected, which will prevent the build up of foam in the air trap or can trigger the next step in the method.

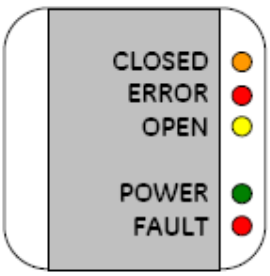
3.7.2.2 Valves

General description

With the exception of the filter housing options that have either one or three manual air outlet valves, all valves are diaphragm valves that are actuated by compressed air. The valve actuators are controlled by an ASi bus.
The inlet and outlet valve configurations are identical. Each valve consists of a valve body, a diaphragm and an actuator. Two valves are combined into a valve block.
Due to their size and weight, the valves for 1" systems are mounted in turnable cradles.

Valve LED indicator lights

The valve LED indicator lights are illustrated below.



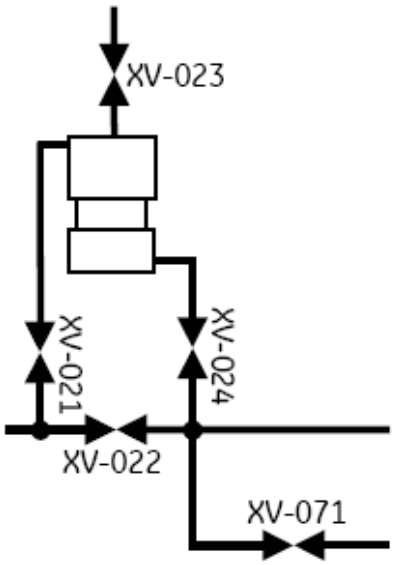
Label	Color	Description (when applicable)
CLOSED	Orange	Steady light: Actuator in closed position
ERROR	Red	Steady light: Programming, sensor or internal error
OPEN	Yellow	Steady light: Actuator in open position
POWER	Green	Voltage on
FAULT	Red	Steady light: Slave address error

Valve default positions

When the system is powered up and connected to compressed air, the default positions for the various valves are given in the table below. If no control signal is present, for example if the mains power is shut off, the valves will revert to ***Closed*** positions.

Air trap valves

The air trap valve blocks are directly connected, either to the optional filter valve blocks or to tubing going to the pre-column air sensor, in order to minimize the dead volume that is caused by connecting valve blocks with tubing.
The layout of the air trap valves is illustrated below.



Valve positions	Open valve(s)
Bypass	XV-022
Inline (Default)	XV-021 + XV-024
Fill	XV-021 + XV-023
Fill_Inline	XV-021 + XV-023 + XV-024
Out_through_drain	XV-021 + XV-024 + XV-071
Drain (No flow)	XV-023 + XV-024 + XV-071

The ***Drain*** valve position is used for example when the air trap is emptied before disassembly or to lower the liquid level in the air trap. The pump(s) must be set to 0.0 l/h when

the ***Drain*** valve position is used.

Instruction	Setting
Valves:AirTrap	*Bypass*
	Fill
	Fill_Inline
	Drain
	Out_through_drain

The instruction ***System:Settings:Specials:AirTrapPauseFunction*** defines if the valve goes back to the default position (Inline) or if it remains in position when the system is set to Pause.

Sample connection valves

The optional sample pump is connected to the sample connection valve, where the feed from System pump A and the optional gradient pump B is also connected, after the air trap and the optional filter. Sample inlet valves are available only on systems that are delivered with a sample pump.
When an inlet valve is open (A, B or Sample inlet valves), the corresponding sample connection valve will also open. The sample connection valves cannot be controlled independently by the operator.

Note:
The alarm for the sample inlet valves must be disabled if the sample pump is disconnected. See Section5.1.5 Final checks, on page 123 for UNICORN settings.

Column valves

The column valve sets (column 1 and the optional column 2) each consist of six valves. Similar to the inlet valve blocks, the blocks are connected directly to each other to enable the shortest possible flow path.

3.7.2.3 Optional components

Extra system pump inlets

Up to eight extra inlets with individually controlled valves can be installed.
This means that ÄKTAprocess is able to manage up to ten individual inlets for system pump A.

Extra system outlets

Up to eight extra system outlets with individually controlled valves can be installed.
This means that ÄKTAprocess is able to manage up to ten individual outlets in total.

System pump B

ÄKTAprocess can be provided with a second system pump. The addition of a second system pump enables ÄKTAprocess to operate as a gradient system.
The B-pump can be provided with up to six individually controlled inlets.
If the System pump B option is selected, an extra flow meter can also be provided to

enable the individual pump flows, as well as the total system flow, to be measured.
The B-pump type is identical to the A-pump.

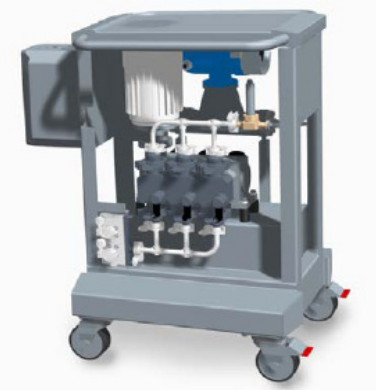
In-line filter

A filter can be installed between the air trap and the column to prevent foreign objects from contaminating the column. Different types of in-line filter are available, including a disposable capsule filter option. Filter housings may be made of steel or polypropylene.

Two columns

ÄKTAprocess can be configured to incorporate a second column. With this option, the ÄKTAprocess can supply two columns, one after the other with mobile phase.

Sample pump



The sample pump allows sample to be injected into the column without the need to use any of the system pumps for this purpose.
There are two optional inlets feeding the sample pump. The sample pump is also provided with an extra pressure meter that protects the system against over pressure.

Extra pressure meter

An extra pressure meter can be installed after the column to accurately measure the pressure drop over the column.

Extra pH meter

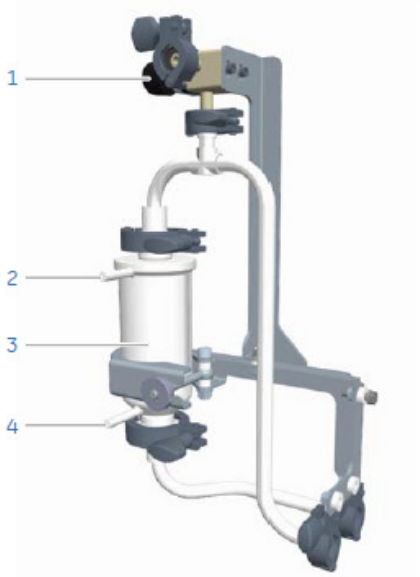
An extra pH-meter can be installed before the column on a gradient system to enable the gradient to be monitored.

Filter valves

The optional in-line filter valve block set is identical to the air trap valve block set. There is also a manual valve for air evacuation, HV-301.
The layout of the filter valves is illustrated in the following diagram.

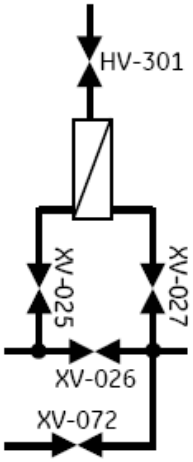
Capsule filter and valves

A disposable capsule filter option may be selected instead of the in-line filter option described above. The disposable capsule filter housing is made of polypropylene and includes two additional manual valves for air evacuation, as illustrated below.



Part	Function
1	Manual valve **HV-301**
2	Outlet to manual valve **HV-303**
3	Capsule filter housing
4	Outlet to manual valve **HV-302**

The layout of the capsule filter valves is illustrated in the following diagram.



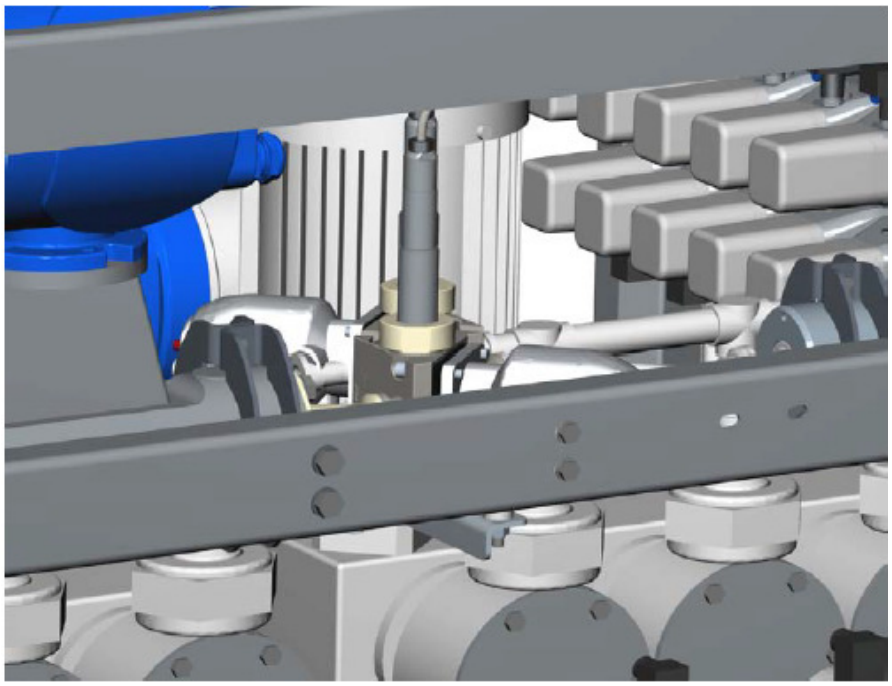
Valve positions	Open valve(s)
Bypass (default)	XV-026
Inline	XV-025 + XV-027
Out_through_drain	XV-025 + XV-027 + XV-072
Drain (No flow)	HV-301 + XV-027 + XV-072

The ***Drain*** valve position is used, for example, when the filter housing is emptied before replacing the filter. The pump(s) must be set to 0.0 l/h when the ***Drain*** valve position is

used.

Pressure control valves (PCV)

ÄKTAprocess can be provided with up to two optional pressure control valves, **PCV-341** and **PCV-342**, as shown in the illustration below.



The function of the PCVs is to protect the system from 'free flow' if the inlets are fed with a higher pressure than 0.2 bar.
The pressure control valve option allows the pressure on the inlets to be regulated and the flow through the system via individual system pumps to be controlled.

ALP2 PCV safety monitoring

If ÄKTAprocess is optionally configured to include a pressure control valve, or valves, the system will also be equipped with an **ALP2** air, level and pressure monitoring system.
The **ALP2** unit protects against exceeding maximum operating pressures by monitoring pressure between the pumps and the PCVs.

CIP / AxiChrom manifold

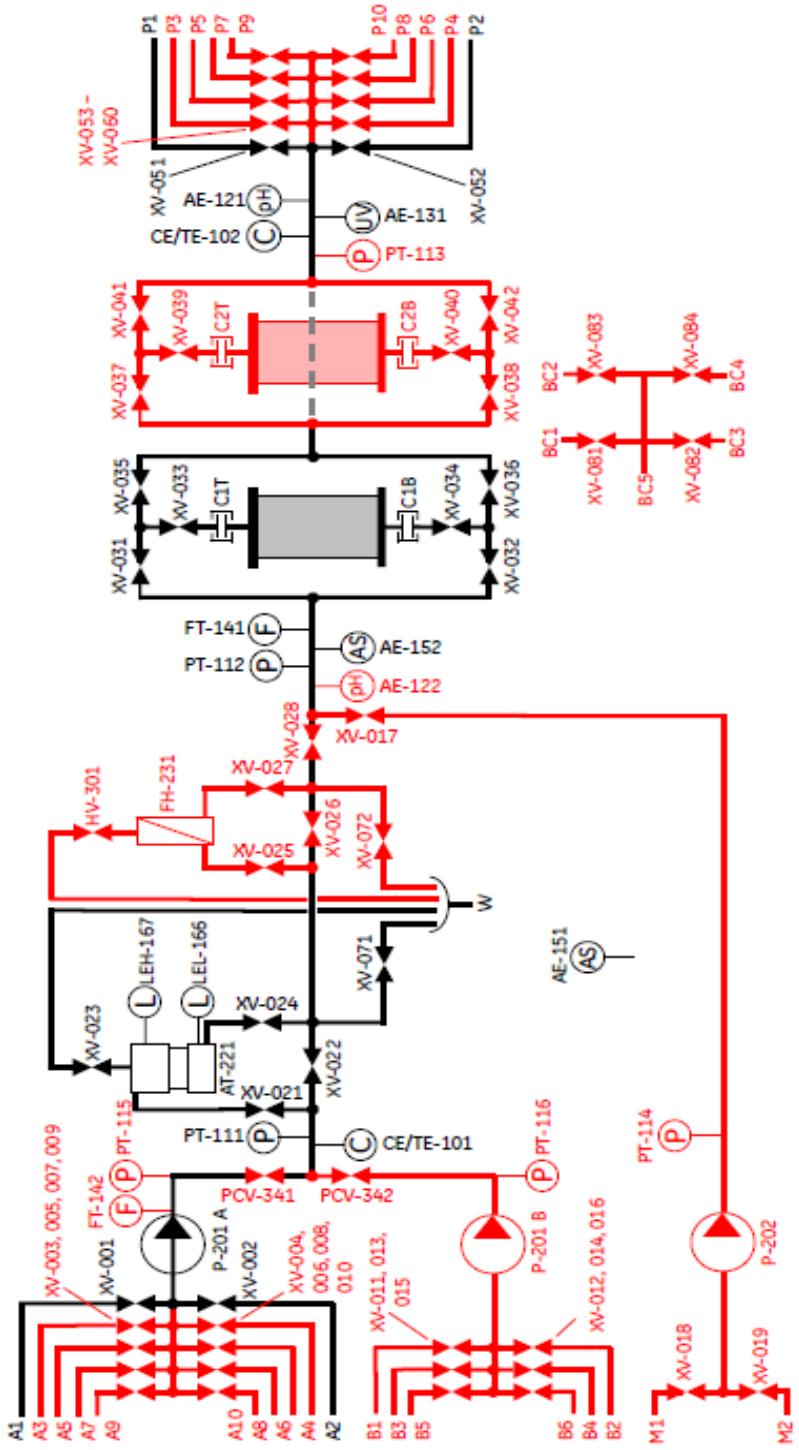
A CIP / AxiChrom manifold with four individually controlled valves enables UNICORN to control CIP with up to four inlets and control processing together with a connected AxiChrom column.
See *Using the CIP/AxiChrom manifold option, on page 139* for more information about connections for CIP. For Intelligent Packing with AxiChrom columns, see AxiChrom manuals for details on connection.

Anybus™ X-gateway

The system can be provided with an optional Anybus X-gateway to enable communication between ÄKTAprocess and the customer network. The signals transferred can both be analog process readings, digital status and handshake signals. The Anybus X-gateway is located inside the electrical cabinet. The interface to the gateway is PROFIBUS Slave through a M12 connector in the bottom of the electrical cabinet.
The Anybus X-gateway copies I/O-data in both directions, thus enabling data exchange

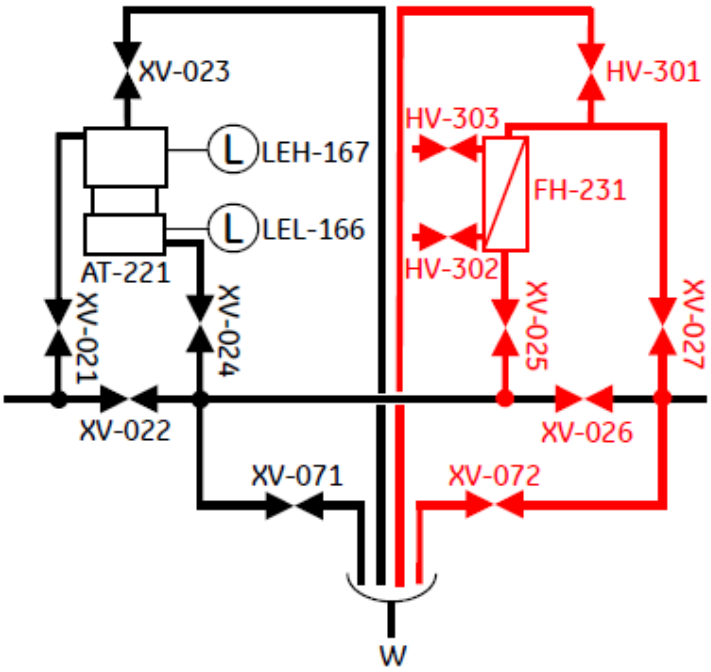
between two optically isolated PROFIBUS networks. The Anybus X-gateway connections can be used for many different applications. See the PROFIBUS Communication Interface documenation for a description of how the different I/O-data is addressed in the memory space.

3.7.2.4 *Flowchart*



Capsule filter option

The illustration below shows the corresponding air trap and filter block alternative section of the flowchart if the capsule filter option is selected.



Note: *Black represents standard components; Red represents optional components*

3.7.2.5 Process components

The following table lists the process components that are shown in the flow chart.

Tag	Function (qty)	Note
1, 2	Outlets	
3 to 10	Outlets	Optional
A1, A2	Buffer A inlets	
A3 to A10	Buffer A inlets	Optional
AT-221	Air trap	
B1 to B6	Buffer B inlets	Part of system pump B option
C1T	Column 1 top connection	

Tag	Function (qty)	Note
C1B	Column 1 bottom connection	
C2T	Column 2 top connection	Part of column 2 option
C2B	Column 2 bottom connection	Part of column 2 option
CIP1 to CIP4	CIP inlets	
CIP C	CIP common inlet	
FH-231	Filter	Option
HV-301	Filter vent valve	Part of filter option, manual
HV-302	Capsule filter bottom manual valve	Capsule filter option only
HV-303	Capsule filter top manual valve	Capsule filter option only
M1, M2	Sample inlets	Part of sample pump option
P-201 A	System pump A	
P-201 B	System pump B	Option
P-202	Sample pump	Option
PCV-341	Pressure control valve, A inlets	Option
PCV-342	Pressure control valve, B inlets	Option
W	Common waste	
XV-001, XV-002	Buffer A inlet valves	
XV-003 to XV-010	Buffer A inlet valves	Optional
XV-011 to XV-016	Buffer B inlet valves	Part of system pump B option
XV-017	Sample connection valve	Part of sample pump option
XV-018, XV-019	Sample inlets valves	Part of sample pump option
XV-021	Air trap inlet valve	
XV-022	Air trap bypass valve	
XV-023	Air trap vent valve	
XV-024	Air trap outlet valve	
XV-025	Filter inlet valve	Part of filter option

Tag	Function (qty)	Note
XV-026	Filter bypass valve	Part of filter option
XV-027	Filter outlet valve	Part of filter option
XV-028	System connection valve	Part of sample pump option
XV-031	Column 1 top inlet valve	
XV-032	Column 1 bottom inlet valve	
XV-033	Column 1 top valve	
XV-034	Column 1 bottom valve	
XV-035	Column 1 top outlet valve	
XV-036	Column 1 bottom outlet valve	
XV-037	Column 2 top inlet valve	Part of column 2 option
XV-038	Column 2 bottom inlet valve	Part of column 2 option
XV-039	Column 2 top valve	Part of column 2 option
XV-040	Column 2 bottom valve	Part of column 2 option
XV-041	Column 2 top outlet valve	Part of column 2 option
XV-042	Column 2 bottom outlet valve	Part of column 2 option
XV-051, XV-052	Outlet valves	
XV-053 to XV-060	Outlet valves	Optional
XV-071	Air trap drain valve	
XV-072	Filter drain valve	Part of filter option
XV-081 to XV-084	CIP / AxiChrom manifold	Option

3.7.2.6 Meters and sensors

The following table lists the meters and sensors that are shown in the flow chart.

Tag	Function	Note
AE-151	Buffer inlet air sensor	Movable
AE-152	Pre-column air sensor	Final check that no air enters the column
AT-121	Post-column pH-meter	
AT-131	Post-column UV-meter	Peak detection
CE/TE-101	Pre-column conductivity meter	Also includes a temperature meter
CE/TE-102	Post-column conductivity meter	Peak detection and CIP-control, also includes a temperature meter
FT-141	System flow meter	Measures the total system flow
LEH-167	Air trap high level meter	
LEL-166	Air trap low level meter	
PT-111	Pre-filter pressure meter	Option
PT-112	Pre-column pressure meter	Guards the column from over pressure, detects clogged column
PT-114	Sample pump pressure meter	Part of sample pump option
PT-115	PCV pressure meter, A inlets	part of PCV option
PT-116	PCV pressure meter, B inlets	part of PCV option

3.7.2.7 Connect a column

Considerations for AxiChrom columns

AxiChrom columns can be packed using ÄKTAprocess and Intelligent Packing.
Small diameter columns, up to 200 mm diameter, use ÄKTAprocess pumps to drive the adapter hydraulically.
Larger AxiChrom columns, 300 mm diameter and above, use an AxiChrom Master connected to the ÄKTAprocess Profibus connector.
See respective AxiChrom column manuals for information on hose connections.
For more details regarding using AxiChrom with ÄKTAprocess, refer to the AxiChrom user manual.

Preparations

The air sensor alarm(s) before the column must be disabled before filling the system/column with liquid (***Alarms:Air_Alarm:Disabled***).

Connect an empty column

Follow the instruction below to connect an empty column to ÄKTAprocess.

Step Action

1 Connect tubing between the system valve marked **COLUMN BOTTOM** and the column bottom.
2 Connect tubing between the system valve marked **COLUMN TOP** and the column top.

Connect a packed column without bypass lines/valves

Follow the instruction below to connect a pre-packed column without by-pass lines/valves to ÄKTAprocess.

Step	Action
1	Connect tubing to the system valve marked **COLUMN BOTTOM**, but do not connect the other end of the tubing to the bottom of the column at this time.
2	Set the system column valves to the ***UpFlow*** position.
3	Using the pump, fill the system with an appropriate liquid for column installation.
4	When the system, including the tubing connected to the column bottom system valve, is filled with liquid, connect the other end of the tubing to the bottom of the column.
5	Connect tubing between the top of the column and the system valve marked **COLUMN TOP.**

Connect a packed column with bypass lines/valves

Follow the instruction below to connect a pre-packed column with bypass lines/valves to ÄKTAprocess.

Step	Action
1	Connect tubing between the column bottom and the system valve marked **COLUMN BOTTOM**.
2	Connect tubing between the column top and the system valve marked **COLUMN TOP**.
3	Set the system column valves to the ***UpFlow*** position.
4	Using the pump, fill the system with an appropriate liquid for column installation.
5	When the system, including the tubing connected to the column, is filled with liquid, stop the pump.
6	Use the manual valves on the column to change from bypass to in-line.

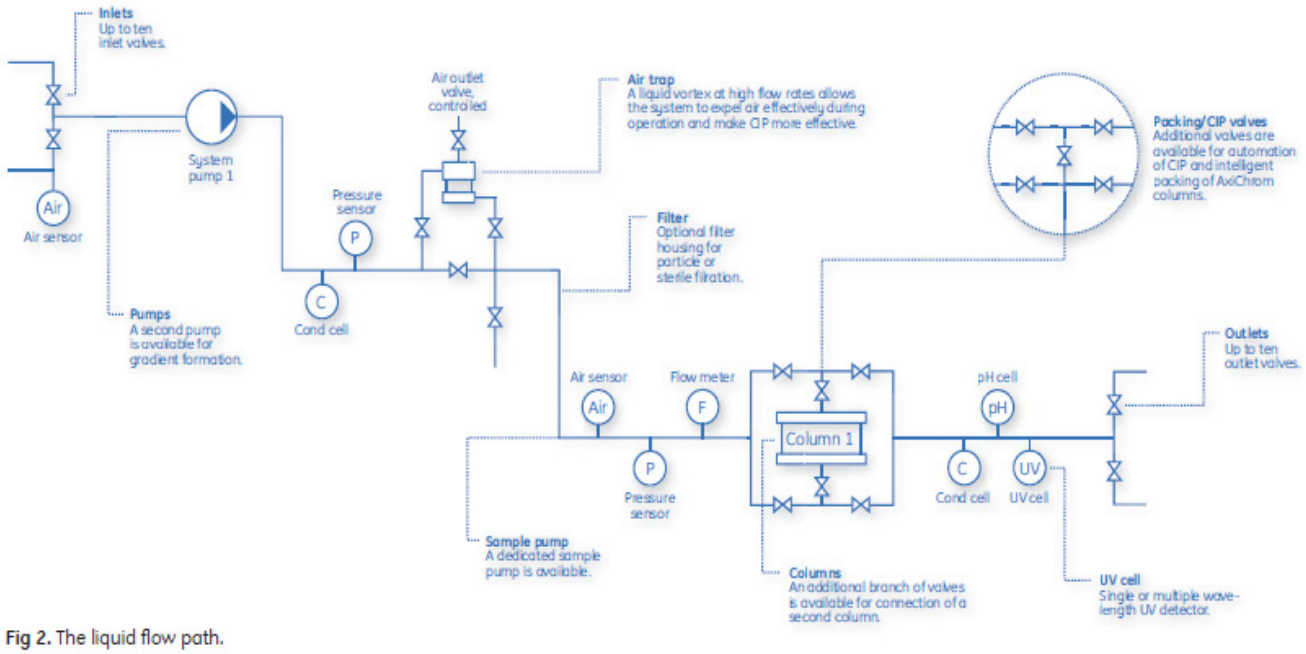


Fig 2. The liquid flow path.

AxiChrom™ 300-1000 columns

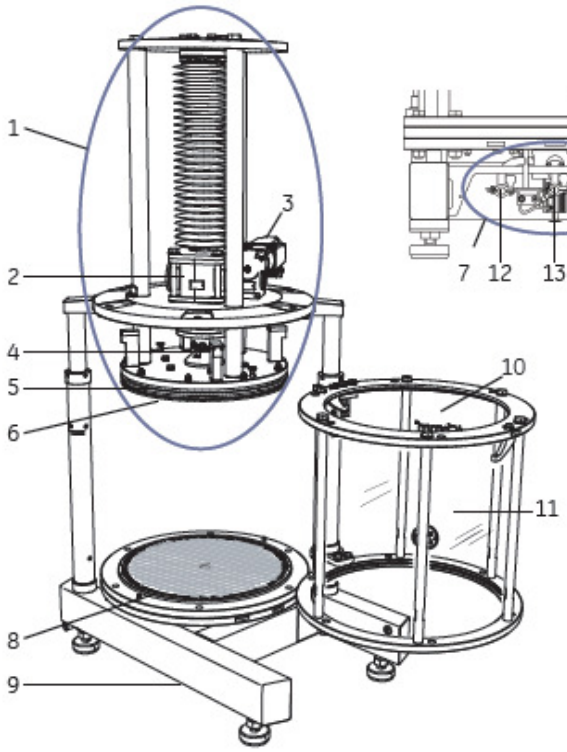
AxiChrom process column family has been designed to deliver reproducible results from process development to production scales. This is facilitated by the innovative Intelligent Packing where UNICORN™ software, ÄKTA™ systems and AxiChrom columns work together to facilitate a convenient operation for packing of the bed via axial compression

The images below show a 600 column (left) and an AxiChrom Master unit (right).

Intended use

The AxiChrom family of process columns has been designed for low pressure chromatographic separation of biomolecules such as proteins, peptides and oligonucleotides in GMP-regulated environments. AxiChrom columns are intended for production use only and should not be used for diagnostic purposes in any clinical or *in vitro* procedures. The columns are not suitable for operation in a potentially explosive atmosphere or for handling flammable liquids. If the columns are used for purposes other than those specified in the user documentation, safe operation and the protection provided by the system may be impaired.

3.7.2.8 Overview of the column and parts



Part	Function	Part	Function
1	Top unit	8	Bottom bed support and distributor plate
2	Worm gear and bellows	9	Column stand
3	Servo motor	10	Process chamber
4	Top mobile phase inlet/outlet	11	Column tube
5	Adapter	12	Slurry inlet
6	Adapter bed support and distributor plate	13	Bottom mobile phase inlet/outlet
7	Media valve assembly	14	Rinse inlet

Inlet and outlet system

- The tubing connection at the Top mobile phase on the adapter is the only liquid

connection at the top of the column.

- At the bottom the center inlet/outlet is the bottom mobile phase.
- The Slurry inlet is connected to the slurry tank from where the media slurry is drawn into the column. Inside the Media valve under the column, the Slurry inlet has a liquid connection with the Rinse inlet.
- The Rinse inlet is used to flush the Media valve, the Slurry inlet and the tube to the slurry tank free from residual media after filling and unpacking the column. When a filling process has ended, the Media valve closes and a pump connected to the Rinse inlet pumps the liquid through the Media valve, and then through the Slurry inlet to the slurry tank.

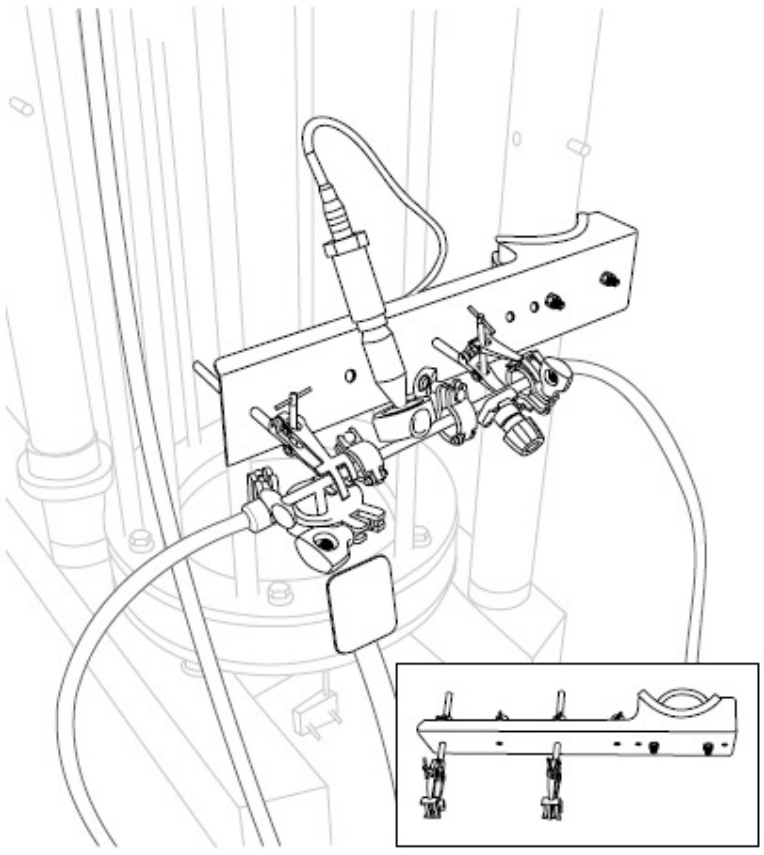
- The Slurry inlet and Rinse inlet have no connection to the Bottom mobile phase flowpath. When the Media valve is open, the Slurry inlet and Rinse inlet lead directly to the process chamber. Liquid in the mobile phases, on the other hand, has to flow through the bed supports to reach the process chamber.

Connection flanges

- **Tri-clamp 25** is used for the 300, 400, 450 and 600 columns.
- **Tri-clamp 50** is used for the 800 and 1000 columns.

Valve holder (accessory)

The valve holder accessory is useful for fixing components to the column assembly, particularly when a number of serially connected valves and sensors are used. The valve holder is clamped on the front stand tube with a U-rod and holds the components with 3-pronged clamps. One valve holder carries two clamps. Different sizes of U-rod are used for AxiChrom 300-600 columns and AxiChrom 800-1000 columns respectively.



Valve holder accessory (inset) and mounted with valves and sensors on front stand tube.

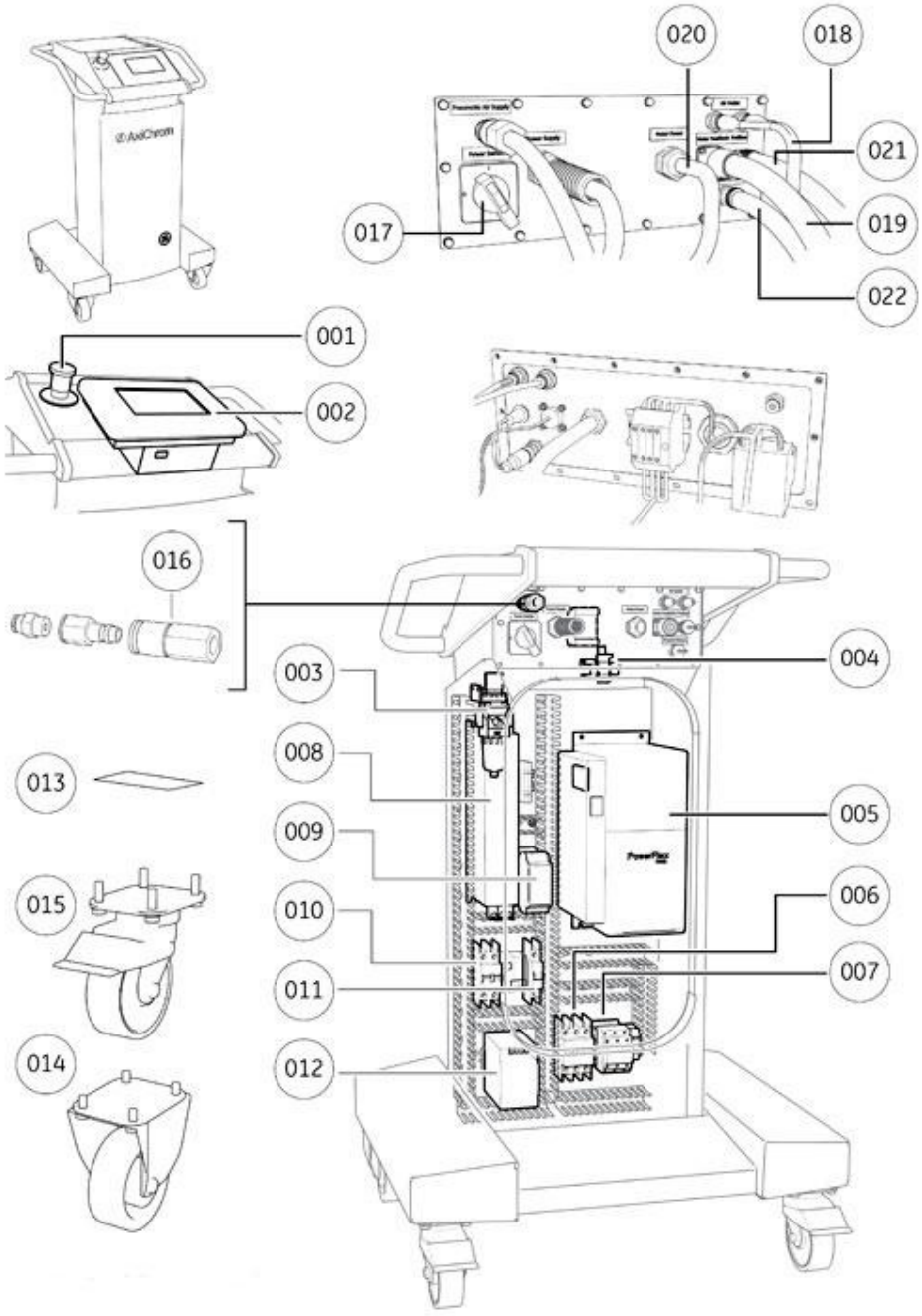
AxiChrom Master

AxiChrom Master is a self-contained operator console featuring interactive guides for work procedures such as packing, unpacking and maintenance. The user interface is a touch screen panel.

The interactive firmware guides the user and reduces the risk of making mistakes. The operator has control over the workflow, and can use manual control for adapter movement and open or close the Media valve.
One AxiChrom Master unit can be used to control up to ten columns (one at a time).

AxiChrom Master specifications

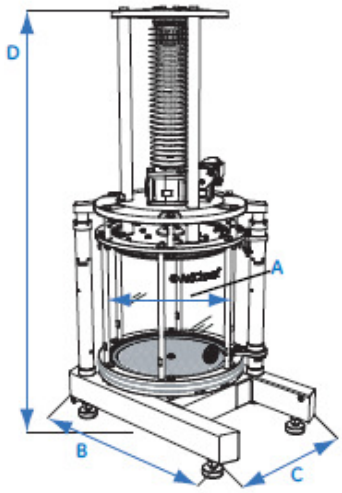
Parameter	Value
Weight	Approximately 73 kg (161 lbs)
Length × width × height	670 × 590 × 1090 mm
Material	Stainless steel, ASTM 316 and ASTM316L (EN 1.4401/1.4436 and EN 1.4404/1.4432/1.4435)



1	Emergency stop switch
2	Display for AxiChrom master
3	Air filter regulator
4	Air solenoid valve
5	AxiChrom Master Control Unit (Spare Part)
6	Circuit breaker

7	Contactor 25A 24V DC
8	Mains filter 3x480 V 16A
9	Safety relay
10	Circuit breaker
11	Circuit breaker 4A, 1-pole
12	Power supply 400 V AC - 24 V DC
13	Wheel gasket
14	Wheel without brake
15	Wheel with brake
16	Air quick connector
17	Mains Switch
18	Air hose kit
19	AxiChrom master encoder cable
20	AxiChrom master motor cable
21	AxiChrom master profibus cable
22	AxiChrom master pressure cable

Weights, volumes and related dimensions of axichrom



Column	300	400	450	600	800	1000
Tube inner diameter (A) (mm)	300	400	450	600	800	1000
Footprint (B×C) (mm)	1110 × 520	1110 × 600	1110 × 620	1180 × 780	1470 × 1080	1720 × 1300
Safety zone (mm)	3200 × 2600	3200 × 2600	3200 × 2700	3200 × 2800	3500 × 3100	3800 × 3300
Column cross section (cm^2)	707	1257	1590	2827	5027	7854
Max bed volume, short/long tube (liters)	21/35	36/63	48/80	85/141	151/251	236/393
Weight of empty column, short/long column, stainless steel bed support (kg)	420/440	460/480	710/760	835/900	2150/2240	2560/2680
Weight of empty column, short/long column, plastic bed support (kg)	414/434	451/471	699/749	818/883	2122/2212	2517/2637

Heights in different operating states

Column	Tube	300	400	450	600	800	1000
Max height [1] (D)	Short [2]	2200		2230	2340	2630	2650
	Long [3]	2720		2750	2860	3150	3170
Height (D) when adapter is at max bed height	Short	1740		1760	1870	2160	2170
	Long	2200		2220	2330	2620	2630
Min height (delivery height)	Short	1450	1460	1480	1590	1880	1890
	Long	1710	1720	1740	1850	2140	2150
Max slurry filling height	Short	2040		2060	2185	2335	2490
	Long	2555		2580	2700	2850	3005
Max operating height (D) during priming	Short	2060		2080	2190	2480	2490
	Long	2580		2600	2710	3000	3010
Max adapter stroke height for filling	Short	570				578	
	Long	830				838	

Operating conditions

Parameter	Value
Maximum operating pressure [1]	4 bar
Operating temperature [2]	2°C to +30°C
Operating pH [3]	1 to 14

3.7.2.9 Materials

Background information

The materials used to manufacture AxiChrom columns have been chosen for their biological and chemical compatibility with the solvents used during operation and cleaningin-place (CIP) procedures. The columns have also been designed to comply with the varying hygienic requirements at the different stages of development and production. Polymer materials in AxiChrom columns in contact with process liquids have been selected for their biological compatibility according to the United States Pharmacopeia (USP) Biological Reactivity Tests, *In vivo* and conform to USP class VI requirements, compliance with Code of Federal Regulations (CFR), Food and Drug Administration, Title 21, Part 177 and being animal free or complies with the conditions in the CPMP Note for Guidance (EMEA/410/01 Rev.2).

Column tubes for AxiChrom 300-1000 columns are available in acrylic plastic or stainless steel. Use and maintenance of AxiChrom columns with stainless steel column tubes differs from that for acrylic plastic column tubes in two major respects:

- Stainless steel column tubes are not transparent, so the bed cannot be observed directly. This affects packing and unpacking procedures.
- Stainless steel column tubes are manufactured in a single piece, with no removable tie rods, top or bottom flanges or corresponding O-rings. This does not affect normal operation or maintenance procedures but results in a spare parts and accessories list that differs from that of acrylic columns.

Parts list and materials

Component	Material	In contact with process stream
Adapter backing plate	Stainless steel ASTM 316 or ASTM S32205	No
Adapter seals and snap ring	UHMWPE (ultra high molecular weight polyethylene)	Yes

Component	Material	In contact with process stream
Bed support	Stainless steel ASTM 316L and ASTM S32205 or PE (polyethylene) or UHMWPE	Yes
Column tube	PMMA (polymethyl methacrylate) or Stainless steel ASTM 316L	Yes
Distributor	PP (polypropylene)	Yes
Dynamic seals	FFPM (full fluorinated propylene monomer) or UHMWPE	Yes
Media valve body	PP	Yes
Static seals	EPDM (ethylene propylene diene monomer)	Yes
Top mobile phase	PP	Yes
Bottom backing plate	Stainless steel ASTM 316	No
Lid	Stainless steel ASTM 316	No
Stand	Stainless steel ASTM 316	No

Chemical resistance

AxiChrom columns are resistant to chemical agents used in protein recovery, including buffer solutions for adsorption, elution and washing, and to solutions effective in cleaning, sanitization and storage. *Table* lists chemicals that may or may not be used with AxiChrom columns. The concentrations listed are not normally exceeded during an operating cycle.

Chemical	Concentration [1]	Time/cycle restrictions	Comments	Operating temperature	CAS no. [2]
Acetic acid	25%	3 h	Cleaning-In-Place (CIP)	2°C to 30°C	64-19-7
Acetone	2%	1 h	Efficiency test	2°C to 30°C	67-64-1
Ammonium sulphate	2 M [3]	5 h	Adsorption	2°C to 30°C	7783-20-2
Benzyl alcohol	2%	12 months	Storage	2°C to 30°C	100-51-6
Ethanol	20%	12 months and max. 0.5 bar	Storage	2°C to 30°C	64-17-5
Ethanol	70% [4]	3 h	CIP	2°C to 30°C	64-17-5
Ethanol/ acetic acid	20%/ 10%	3 h	CIP	2°C to 30°C	64-17-5/ 64-19-7
Guanidinium hydrochloride	6 M [5]	5 h	CIP	2°C to 30°C	50-01-1
Hydrochloric acid	0.1 M (pH = 1) [6]	1 h	CIP	2°C to 30°C	7647-01-0
Isopropanol	30% [7]	1 h	CIP	2°C to 30°C	67-63-0

Chemical	Concentration [1]	Time/cycle restrictions	Comments	Operating temperature	CAS no. [2]
Phosphoric acid	5%	8 h	For passivation of stainless steel bed supports	2°C to 30°C	7664-38-2
Sodium chloride	0 to 3 M [3, 6, 8]	3 h	Purification, CIP	2°C to 30°C	7647-14-5
Sodium hydroxide	1 M (pH = 14)	24 h, room temp. to 30°C	CIP	2°C to 30°C	1310-73-2
Sodium hydroxide	0.01 M (pH = 12)	12 months	Storage	2°C to 30°C	1310-73-2
Sodium hydroxide/ ethanol	1 M/ 20%	3 h	CIP	2°C to 30°C	1310-73-2/ 64-17-5
Sodium sulphate	1 M [3]	3 h	Adsorption	2°C to 30°C	7757-82-6
Urea	8 M [3]	5 h	Purification, CIP	2°C to 30°C	57-13-6
Commonly used aqueous buffers for chromatographic use	10 to 250 mM, pH 2 to 10	24 h	Equilibration, adsorption, elution	2°C to 30°C	