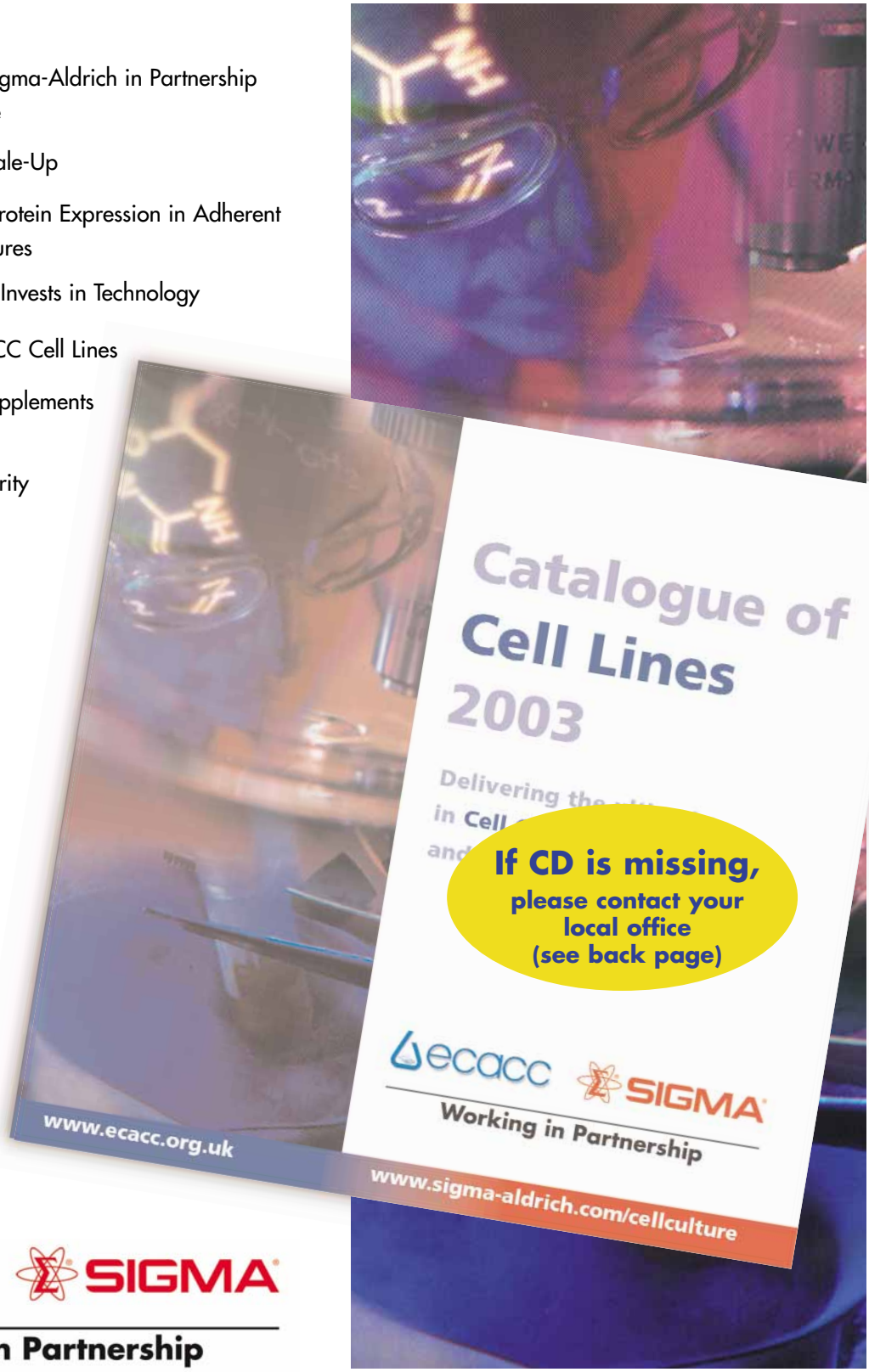
NEWS UPDATE
SPRING 2003

Cell Culture

Contents



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Working in Partnership

ECACC and Sigma-Aldrich Partnership for Cell Culture

ECACC (The European Collection of Cell Cultures)
and Sigma-Aldrich have formed

a working partnership

to deliver the ultimate in cell culture products and services

ECACC

ECACC is a world leader and recognised expert in the maintenance, cultivation and distribution of authenticated cell lines.

Sigma-Aldrich

Sigma-Aldrich is a world leader in the development, optimisation and manufacture of cell culture media and reagents.

Together, we offer a comprehensive range of cell culture products and services available to satisfy your cell culture needs.

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Easy to Order ECACC Cell lines

You can order ECACC Cell lines through your local Sigma-Aldrich office like any other Sigma-Aldrich product.

Reliable Delivery Across Europe

Up to 5 days or less from placing your order.

Serum Free Adaptation

A number of cell lines are now available pre-adapted to Sigma-Aldrich's serum-free or animal component-free speciality media.

Guaranteed Mycoplasma Free

Quality control of all cell lines to guarantee mycoplasma free culture cell banks produced by ECACC.

Custom Capability

Customised media formulation for large scale culture is a speciality of Sigma-Aldrich. From optimised formulations through animal components free media to specialised packaging are manufactured under cGMP conditions.

Technical Support Locally

Call ECACC directly or your local Sigma-Aldrich office for technical support and advice.

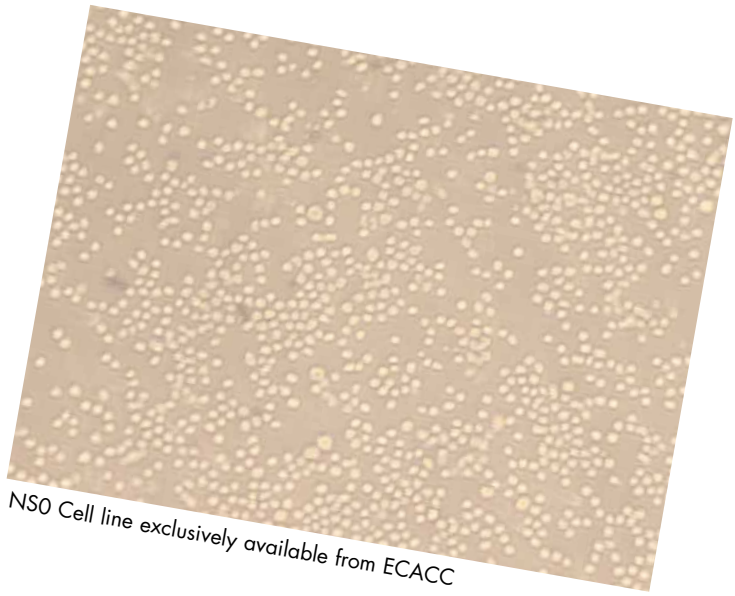
ECACC • TEL: +44 (0) 1980 612512 • FAX: +44 (0) 1980 611315 • EMAIL: ecacc@camr.org.uk

ECACC and Sigma-Aldrich Partnership for Cell Culture

Cell Lines Supplied with Optimised Media

It is important that cells are cultured in the optimum environment. That is why most ECACC cell lines you purchase will come with a FREE 500 ml bottle of the compatible Sigma-Aldrich medium to which the cell line has become acclimatised.

Since most ECACC cells are grown in Sigma-Aldrich media it makes sense to maintain the same conditions. To help you revive cells quickly and successfully we recommend that you use media supplied. Simply add serum and other supplements as required.



NS0 Cell line exclusively available from ECACC

An Environmentally Friendly and Durable Container for Shipping ECACC Cell Lines and Sigma-Aldrich Liquid Media

How to Order

Simply order the ECACC cell line you need from your local Sigma-Aldrich office (see back cover for details of your local office), or direct from ECACC.

The cells and media will be despatched directly to you in an **environmentally friendly, insulated pack.**

Separate compartments for cells and media ensures both arrive in perfect condition ready for use.

Contact information

Register to continue to receive the ECACC Sigma-Aldrich Cell Culture Newsletter and request further information on any products and services featured in this issue using the reply card enclosed.

Visit the Sigma-Aldrich web site at sigma-aldrich.com/cellculture for products information on-line.

Visit the ECACC web site for further details on cell lines and services available at ecacc.org.uk

Scale-Up Using the Celligen™ Fixed-bed Bioreactor

The use of a Celligen™ Fixed-bed Bioreactor in the Production of Alkaline Phosphatase (AP) from Recombinant CHO Cells using Sigma-Aldrich CHO, Protein-Free Medium.

Recently, ECACC has added a Celligen™ fixed-bed Bioreactor (New Brunswick Scientific (UK) Ltd) to its scale-up and process development. This is in addition to it's range of standard stirred tank bioreactor (1L to 100L). As part of an on-going commission to test the effectiveness of the Celligen™ fixed-bed bioreactor, the process development group at ECACC used the AP recombinant CHO cell line from Sigma-Aldrich to demonstrate the capabilities of the Celligen™ fixed-bed bioreactor.

The Celligen™ fixed-bed bioreactor (Figure 1) has a proven record over many years in the production of monoclonal antibodies. More recently, fixed-bed bioreactors have been successfully used in the production of secreted proteins from recombinant cell lines.

ECACC is now in a position to offer the services of its' pilot-scale Celligen™ fixed-bed bioreactor for non-GMP production runs, and as a process development tool.

Fixed-bed perfusion offers a number of advantages:

- Constant and controllable pH, temperature, dissolved oxygen (DO) and constant metabolites
- The product is removed from the bioreactor as soon as it is secreted. This is a distinct advantage if the product is labile or sensitive to degradation by proteolytic enzymes
- The cells are protected from shear forces in the matrix of the Fibracell™ packed bed.

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In this study, ECACC's 1.4L pilot scale Celligen™ fixed-bed bioreactor was used.

The cell line, reagents and AP assay kits used in this study were provided by Sigma-Aldrich (Poole, UK).

The innoculum was grown using conventional cell culture techniques in Sigma-Aldrich CHO, Protein-Free Medium (Product Code: C5467) supplemented with 4mM L-glutamine and puromycin as a selective agent to maintain a secreting population of cells. Puromycin was omitted in the production run.

The Celligen™ fixed-bed bioreactor was seeded with 1 x 10^9 of the AP recombinant CHO cells in 1L of CHO Protein-Free Medium in a packed bed of 100g of Fibracell discs. Daily samples were taken from the reactor vessel to ascertain AP productivity, glucose and lactate levels. The bioreactor was left for approximately 66 hours post-seeding before perfusion commenced. The perfusion rate was adjusted daily in response to the glucose consumption rate and to maintain lactate at a sub-inhibitory level. Product (perfusate) was collected into autoclave-sterilised carboys and chilled to +4°C using an insulated ice bath.

The DO and pH in the bioreactor was controlled by a 4 gas mix (O_2, N_2, CO_2 and compressed air). Later in the run, the periodic addition of sodium bicarbonate was required to prolong pH control. The Celligen™ controller in conjunction with AFS Biocommand software (New Brunswick Scientific (UK) Ltd.) constantly monitored reactor conditions. The bioreactor was operated for 309 hours (13 days) before the run was electively terminated.

The summary table 1 shows the AP, glucose and lactate level in samples taken at daily intervals from the reaction; and the perfusion rate of the medium. From these figures we were able to calculate the glucose concentrate rate of the system and the AP productivity in both units per hour and units per litre. The volume of product per day was measured and from this the daily yield of AP was estimated (Figure 2).

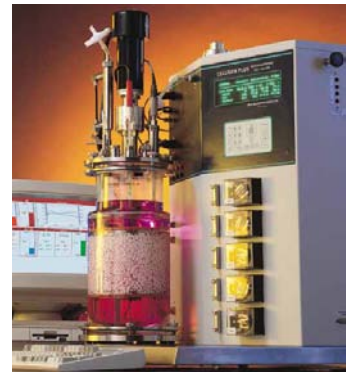

Figure 1. Celligen™ fixed-bed bioreactor

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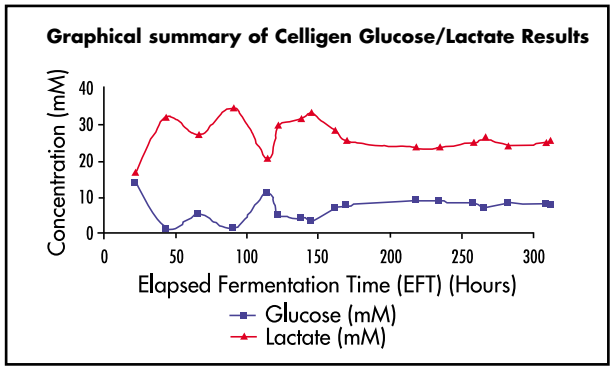
Scale-Up Using the Celligen™ Fixed-bed Bioreactor

Results

Time (hours)	AP (units/ml)	Glucose (mM)	Lactate (mM)	Glucose Consumption (g/day)	Perfusion ml/hr	Perfusate (AP) Productivity (units/hour) ($x10^3$)	Perfusate (AP) Units Per Litre ($x10^3$)	Litres Produced per day	Daily Yield (units) ($x10^4$)
21.62	16.22	14	16.9	3.02	0	0.0	0.0	0.0	0.0
43.05	44.90	1.32	32.4	3.6	0	0.0	0.0	0.0	0.0
65.87	45.10	5.25	27.3	4.5	65	2.93	45.1	1.56	7.04
90.13	67.80	1.5	34.8	8.71	77.7	5.27	67.8	1.86	12.6
113.95	31.03	11.3	20.7	8.5	188	5.83	31.00	4.51	14.0
121.78	36.32	5.08	30	15.7	188	6.83	36.3	4.51	16.4
137.85	29.49	4.29	31.7	16.85	187.5	5.53	29.5	4.50	13.3
144.53	29.69	3.45	33.4	18.04	187.5	5.57	29.7	4.50	13.4
161.42	21.44	7.07	28.6	21.33	292	6.26	21.4	7.01	15.0
169.2	22.72	7.97	25.5	21	292	6.63	22.7	7.01	15.9
217.86	15.01	9.17	23.7	21.8	326	4.89	15.0	7.82	11.7
234.05	12.46	8.97	24	21.2	308	3.84	12.5	7.39	9.21
258	11.32	8.3	25	26.21	365	4.13	11.3	8.76	9.92
265.37	11.05	7.3	26.5	28.54	365	4.03	11.1	8.76	9.68
282.27	8.57	8.64	24.5	20.74	304	2.61	8.57	7.30	6.26
306	7.64	8.24	25.1	25.75	358	2.73	7.64	8.59	6.56
309.08	7.57	7.9	25.6	24.47	358	2.71	7.57	8.59	6.50
Mean units/ day	24.6						Total	92.68 Litres	1.68E+06 Units
Final Concentration of AP in Pooled Harvest									18 Units/ml

Table 1. Summary of Celligen™ Run



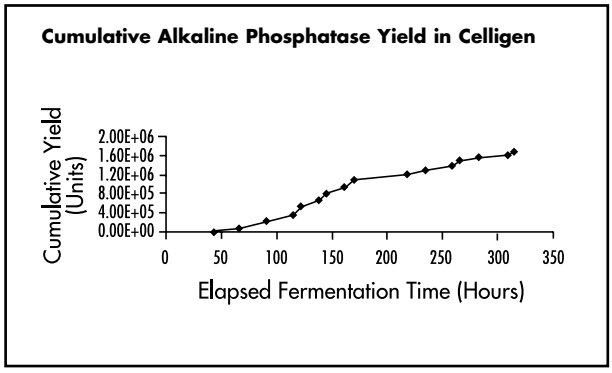


Figure 2.

Conclusions

The Sigma-Aldrich CHO Protein-free medium supported good cell growth achieving high cell densities along with good viability and productivity. The pilot scale Celligen™ (1.4L) can provide the equivalent yield of AP from the rCHO AP cell line as 136 x 300ml roller bottles (data not shown). The Celligen™ has the advantage that the reactor conditions can be maintained at steady state of dissolved oxygen and pH, and of critical metabolites such as glucose and lactate.

The Celligen™ fixed-bed bioreactor is scalable and suitable for use in cGMP manufacture. Fewer manipulations are required as compared to roller bottles, therefore contamination risk is reduced and the process is less labour intensive.

The system employed in this case was not optimised, and we are confident that with further work and trials, the yield could be increased and output sustained for a longer period.

Acknowledgements
ECACC appreciates the collaborative commitment between its team members to provide high quality through-put in the required time-scale.

Information Request 101

Recombinant Protein Expression

New Advanced Protein-Free, Animal Component-Free Medium for Recombinant Protein Expression in Adherent CHO Cell Cultures

Abstract

As more and more recombinant proteins are being used as therapeutic agents, the methods employed in their production are coming under increasing regulatory scrutiny. One of the areas of regulatory concern is the presence of animal-derived components in the media used to grow cells for recombinant protein expression. A CHO Protein-free Animal Component-free Medium (Sigma C5467) was previously developed to support suspension culture and to achieve the desired recombinant protein expression. In order to extend the usage of this medium to the adherent cell culture systems, such as roller bottles and fluidised bed bioreactors, we recently developed a CHO Protein-free Animal Component-free Attachment Medium (Sigma C8730).

Our data indicates that recombinant CHO cells growing in T-flasks or roller bottles with this attachment medium exhibit similar cell growth and a higher level of recombinant protein expression as compared to cells grown in DME/F12 supplemented with 10 % v/v foetal bovine serum (FBS). Moreover, CHO cells growing in suspension culture with Sigma C5467 medium can be directly transferred to T-flasks or roller bottles with C8730 medium for a short-term adherent culture without a pre-adaptation step. Besides CHO cells, various cell lines including HEK293, Vero and C127 can be maintained as adherent cultures in the Sigma C8730 medium with some modification and supplementation with appropriate recombinant growth factor(s). This observation strongly indicates that this new medium might be a good basal medium for any customers' specific cell line grown as adherent cultures.

Finally, the performance of this medium (Sigma C8730) for CHO single cell colony isolation for the selection of new recombinant protein expressing cell lines is contrasted with serum-containing media commonly used for this procedure.

Introduction

Chinese Hamster Ovary (CHO) cells are one of the most frequently used cell lines for the expression of recombinant proteins that require post-translational modification to express full biological function. CHO cells used for large-scale production of recombinant proteins are typically grown in suspension cultures using animal serum-supplemented medium. However, animal serum presents several well-documented problems for a biopharmaceutical manufacturers of therapeutic agents. This has led to the development of serum-free media, many of which contain proteins and/or protein hydrolysates from animals and plants. Since pharmaceutical and biopharmaceutical companies have developed many recombinant proteins as therapeutic agents, the methods used in their production are coming under increasing regulatory scrutiny. One of the main areas of concern is inclusion of animal-derived components in medium to grow cells for recombinant protein expression.

Sigma-Aldrich Corporation has developed a series of serum-free, protein-free animal component-free and chemically defined media for suspension culture of CHO cells to meet the various needs of biopharmaceutical industry. A CHO Protein-free Animal Component-free Medium (PF-AF)(Sigma C5467) has been developed to support the suspension culture of CHO cells and to achieve the desired recombinant protein expression. This CHO PF-AF medium contains recombinant human insulin, plant hydrolysates, and proprietary iron chelators. All other components are also of non-animal origin, including amino acids, vitamins, fatty acids and surfactants. In order to extend the usage of this medium to adherent cell culture systems, we recently developed a CHO Protein-Free Animal Component-Free Attachment Medium (Sigma C8730). This medium contains no pluronic and extracellular matrix (ECM) proteins. In this report, the performance of the new attachment medium (Sigma C8730) on adherent cultures of CHO cells and the expression of recombinant protein are presented.

Materials and Methods

Cell Lines. CHO K1 cells, Vero, HEK293 and C127 cells were used. CHO cell line 1 expressing a proprietary recombinant antibody was transferred from a customer to Sigma for custom medium development and optimisation.

Cell Culture and Cell Growth Assays. Cells growing in DMEM/F12 + 10% (v/v) FBS were trypsinised and then washed once with Hank's Balanced Salt Solution (HBSS). Then cells were seeded into T-flasks or roller bottles at a 25 % cell seeding density with Sigma C8730 attachment medium or other media. The speed for roller bottles is 0.5 rpm. The spent medium samples were collected everyday for the analysis of recombinant IgG production. The attached cells were counted by haemacytometer to determine cell growth. For single cell colony formation assays, 250 CHO cells were plated in a duplicate set of 100 mm culture plates with DME/F12 + 10% (v/v) FBS. Two days later, the medium was replaced with (Sigma C8730 on one set of plates.

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Recombinant Protein Expression

Single cell colonies were stained by crystal violet.

Quantitation of Recombinant Humanised IgG. The IgG secreted into the medium was measured by HPLC (Waters 2690 HPLC Millipore, MA) using a protein-G affinity column.

Results and Discussion

Sigma C8730 CHO PF-AF attachment medium was designed to expand the applications of Sigma C5467 CHO PF-AF medium. This new medium supports the adherent culture of CHO K-1 cells in T-flasks, roller bottles and micro-carrier beads with few detached cells (Figure 1 B, C and D). CHO cells grown in C8730 medium show a smaller cell size and more epithelial cell-like morphology as compared to cells growing in serum containing medium (Figure 1A). Additionally, adherent cultures of CHO cell line 1 in T-flasks with Sigma C8730 medium reached a lower maximum density, as compared to cells grown in DME/F12 with 10% (v/v) FBS (Figure 2). With the majority of cells still attached, some cells detached from the flask and grew in suspension in the late culture. However, a higher level of recombinant protein expression was observed in the culture with C8730. Sigma C8730 medium also supports similar adherent cell growth and IgG production of CHO cell line 1 cultured in roller bottles, as compared with that in T-flasks (Figure 3).

CHO Attachment Medium

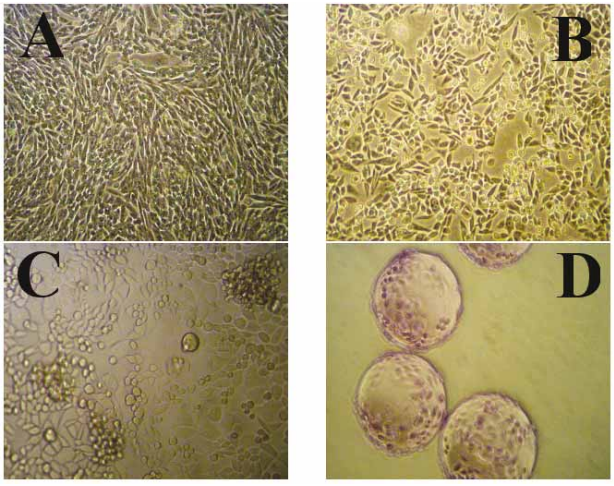


Figure 1. Adherent Cultures of CHO-K1 Grown in Sigma C8730 Medium on T-Flasks, Roller Bottles and Micro-Carrier Beads. Adherent cultures of CHO-K1 cells in DME/F12 with 10% v/v FBS were used on this study (pictures taken on day 3 of culture).
A) CHO cells in T-25 Flasks with DME/F12 with 10% v/v FBS.
B) CHO cells in T-25 flasks with C8730.
C) CHO cells in Roller bottles with C8730.
D) Micro-carrier beads with CHO cells in C8730, cells were visualised by crystal violet staining.

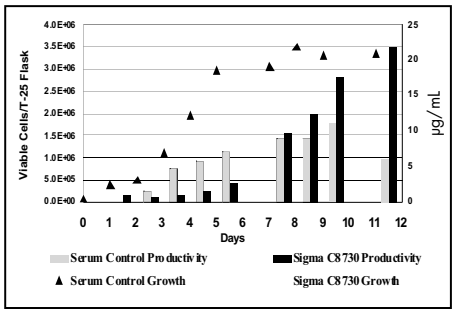


Figure 2. Comparison of Cell Growth and IgG Production with Sigma C8730 and DME/F12 + 10% FBS in T-Flask. CHO cell line 1 grown in DME/F12 + 10% FBS were used for this study.

Sigma C8730 medium also supports the attachment of various cell lines, such as Vero, HEK293 and C127. However, further modifications of this medium, such as supplementation with appropriate growth factors, are required to promote the growth of those attached cells. Interestingly, CHO cells previously grown in suspension with Sigma C5467 medium also can be directly transferred to T-flasks and then cultured with C8730 medium without pre-adaptation. After 6-7 days of culture in Sigma C8730, some cells were found that detached from the surface of flasks and grew in suspension.

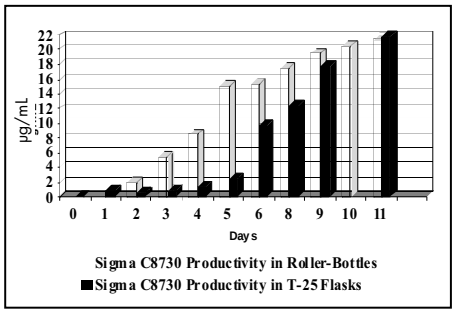


Figure 3. Comparison of IgG Production of CHO Cell Line 1 in Roller Bottles and T-Flasks CHO cells growing in Sigma C8730 PF-AF Attachment Media.

Finally, single cell colony formation of CHO K-1 cells can be achieved by using Sigma C8730 medium after an initial 2 days of culture in serum containing medium. This observation strongly suggests that C8730 medium is useful for the selection of recombinant CHO cell clones after transfection. The utilisation of C8730 instead of serum containing media to select recombinant protein expressing cell clones will help to quickly identify cell clones with maximum performance in C5467 protein free suspension medium without the need to go through a protein free adaptation procedure.

Acknowledgements

We would like to thank all that contributed to the research and development of the CHO media product line at the Sigma-Aldrich R&D St. Louis facility. Thanks also to Bruce Lehr, Sigma-Aldrich cell culture marketing manager, for critical review of this manuscript.
KAO, J. Ross, A. Albee, D. Goodnight, B. Fuhr and M. Caple.

References

Merten, O.W. Safety issues of animal products used in serum-free media Dev Bio Stand. 99:167-179. Review, 1999. Bjare, U. Serum-free cells culture. Pharmac.Ther. 53: 355-374, 1992.

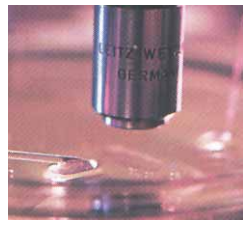
Sigma-Aldrich Invests in Technology

10 Years Experience in Medium Development and Scale Up

Sigma-Aldrich invests in technology, facilities and personnel to make it a world leader in the development, scale up and manufacture of animal-component free media for use in biopharmaceutical production.

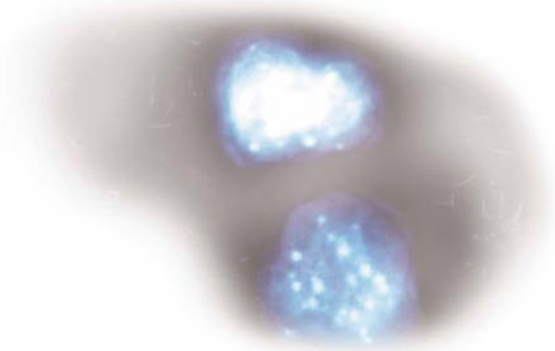
Research and Development

In mid-1998, Sigma-Aldrich commissioned a new $25 million cGMP cell culture media manufacturing facility in St. Louis and completed in early 2003 a $12 million upgrade to its cGMP manufacturing facility in Irvine, Scotland. Both facilities are FDA registered and have obtained ISO certification. In November 2001, we opened a $55 million Life Sciences R&D Center, and built one of the largest cell culture R&D departments in the industry. With these resources, our R&D group has developed more than 100 different serum-free media in support of a wide variety of customer applications within the biopharmaceutical industry in the past three years.



Investment in your Future Research

These investments allow us to work closely with our customers in support of their individual projects.



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We have developed Design of Experiment (DOE) techniques to speed medium development, eliminate animal-derived components, and produce robust, optimised formulations for individual customer clones.

We rely on our expertise in DOE techniques, and use our tremendous analytical resources to perform conditioned medium analysis including amino acids, vitamins and fatty acids, to speed product development. These analytical resources are also critical to raw material qualification and characterisation to ensure consistency of the developed product. We also use these capabilities for assay development and stability studies in support of customers for various regulatory filings.

Verification of ECACC's cultivated cell lines.

New Product Development

By fully utilising our capabilities in medium design, we have introduced new products for the culture of these cell types:

- CHO
- Hybridoma
- Per. C6®
- NS0
- HEK 293

All of these products are completely animal-component free formulations. The NS0 medium is formulated with the world's only synthetic cholesterol, Synthechol™, an exclusive Sigma-Aldrich development. Additionally, we have introduced new media for the culture of haematopoietic stem cells (Stemline™) and continue to work actively in the stem cell arena.

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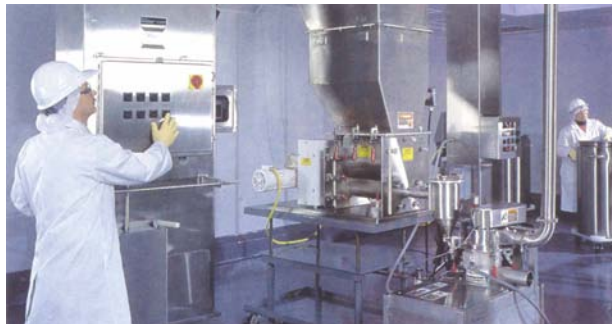
Sigma-Aldrich Invests in Technology

New Products from Sigma-Aldrich

Our newest products include a CHO medium specifically for DHFR- clones (Product No. C8862), that is animal-component free and complements our current highly-successful animal component free CHO medium (Product No. C5467). The new DHFR- medium is specifically very good for DG 44 parental cells.

We will be introducing a novel CHO medium optimisation kit (Product Code CH0001). This new kit allows the user to screen six widely variant, animal-component fee CHO media with the user's clones. Two of these media are completely chemically defined. The kit further provides the user instructions and tools to do additional medium mixing experiments to arrive at an optimised formula for the specific clone. This is the first of its type on the market and is available only from Sigma-Aldrich. The kit is ideal for users who want to do some of their own optimisation work and who would like to learn DOE techniques. Sigma-Aldrich R&D is available for additional consultation with purchasers of this kit.

Visit sigma-aldrich.com/cellculture for further details



Scale-up Facilities

All Sigma-Aldrich media are capable of scale up. We have batch capacity for blended powders utilising our unique Air Classifier Milling technology powders of 4000 Kg using sterile liquid capacity to 10,000 L. Rigorous QC testing ensures lot-to-lot consistency and performance. Sigma-Aldrich understands the regulatory issues and our products are accompanied by full documentation. Custom packaging requirements are offered as part of our service. Whenever you need to develop, optimise, scale up or outsource manufacturing of your cell culture medium, you need to consider the expertise and experience Sigma-Aldrich can offer.

To find out more about how our cell culture capabilities can benefit your organisation, please contact your local Sigma-Aldrich office or representative.

Our Air Classifier Mill automatically transfers milled product to the blender in a continuous, closed process, to ensure consistency.

Custom Cell Culture products/services – the Sigma-Aldrich advantage:

- Over 20 years proven history in cell culture manufacturing and product development
- Dedicated cell culture R & D experts with state-of-the-art facilities
- Global cGMP manufacturing and distribution sites
- Primary manufacturer of most of the key components in cell culture products
- Capabilities in large-scale aseptic and sterile processing of liquids and powders
- Our exacting QA/QC procedures and documentation supports your regulatory requirements

Information Request 103

Per C6 is a registered Trade Mark of Crucell, Netherlands.

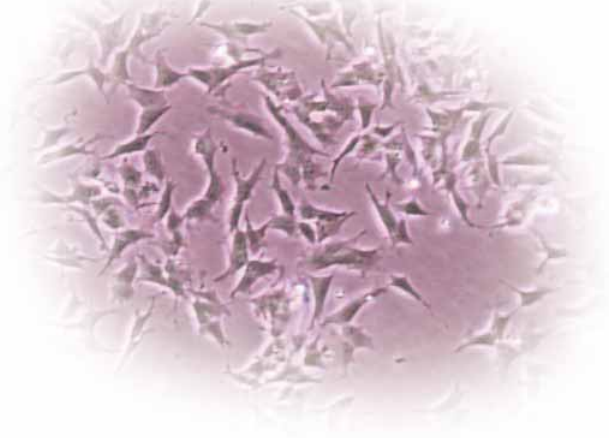
Exclusive Cell Lines from ECACC

The ECACC Cell Line Collection- providing an expanding and exclusive resource for research

ECACC is one of the largest cell culture collections in the world and is constantly striving to provide a broad range of high quality products. In 2002 we added 39 new cell lines to the number of cell lines that are exclusive to our collection and our aim is to do the same this year. In addition to the new cell lines at present there are over 250 cell lines in the ECACC General Collection which are exclusive to ECACC. A list of the 40 most popular of these is listed opposite.

One of ECACC's objectives this year is to expand and broaden the range of cell lines available to you. A recent customer survey enabled us to find out what YOU wanted to see in YOUR culture collection. We were very encouraged by your response and will be in touch shortly with those of you who expressed a desire to deposit cell lines within the collection. If there is a cell line you would like to see in our catalogue, why not get in touch with us and we will consider obtaining it for the collection. At ECACC we are constantly working to add new cell lines.

Contact ECACC by email: ecacc@camr.org.uk



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Popular Cell Lines available exclusively from ECACC

Accession No.	Cell Line Name	Description
88113007	ML-1	Human acute myeloblastic leukaemia
93072111	COLO 720 E	Human andenocarcinoma
99040201	ISHIKAWA	Human asian endometrial adenocarcinoma
95041316	RPMI 8866	Human B lymphocyte
86030402	1321N1	Human brain astrocytoma
96262201	OE21	Human caucasian oesophageal squamous cell carcinoma
93112520	A2780ADR	Human caucasian ovarian carcinoma
93052621	COLO 741	Human colon carcinoma
96121229	tsA201	Human embryonal kidney SV40 transformed
98032302	ISHIKAWA (HERAKLIO) 02ER	Human endometrial adenocarcinoma
95091902	FLYRD18	Human fibrosarcoma (retroviral packaging cells)
94060901	FTC-133	Human follicular thyroid carcinoma
94060902	FTC-238	Human follicular thyroid carcinoma
99072808	F-36P	Human leukaemia, myelodysplastic syndrome
92013101	MTC SK	Human medullary thyroid carcinoma
90071807	KU-812	Human myelogenous leukaemia
93112519	A2780	Human ovarian carcinoma
93112517	A2780cis	Human ovarian carcinoma
85073102	OAW42	Human ovarian tumour epithelial
85101601	OAW28	Human ovarian tumour epithelial
92030501	K1	Human thyroid carcinoma
90011610	Nthy-ts1	Human thyroid follicular mutant epithelial
90071809	CYNOM-K1	Monkey cynomologus skin
88032902	J558L	Mouse BALB/c myeloma
95011018	CGR8	Mouse embryonic stem cell
85110503	NS0	Mouse myeloma
92090903	ND7/23	Mouse neuroblastoma x rat neurone hybrid
92090904	ND8/34	Mouse neuroblastoma x rat neurone hybrid
92090907	ND15	Mouse neuroblastoma x rat neurone hybrid
90030912	Vx2	Rabbit tumour
91100409	OX-61	Anti rat CD26
84112002	W3/25	Anti rat CD4
84112014	OX-26	Anti rat transferrin receptor, CD71
84112008	OX-7	Anti thy – 1.1
94101451	AT-3.1	Rat dorsal prostatic adenocarcinoma
94101453	G	Rat dunning R3327 prostate adenocarcinoma
94101449	AT-1	Rat dunning R-3327, dorsal prostatic adenocarcinoma
89042701	Fao	Rat hepatoma
97041101	NB2-11	Rat lymphocyte

For pricing of cell lines use the CD attached to this newsletter or visit www.ecacc.org.uk

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ECACC Cell Lines

New for 2003 Protein Free Adapted Cell Lines

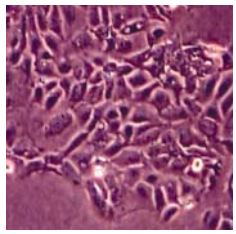
A series of cell lines adapted to growth in protein-free cell culture medium are planned for 2003. Last year saw the launch of our very popular CHO PF cell line. (Chinese Hamster Ovary – Protein Free). This was adapted at ECACC in collaboration with Sigma-Aldrich who provided a specially formulated protein free growth medium and a methylcellulose based medium for cryopreservation. ECACC has begun development work on the adaptation of MDCK, MDBK, HEK 293 and NS0 cells to growth in protein free medium. Look out for the launch of these popular cell lines on our website **www.ecacc.org.uk** or further editions of this newsletter throughout the year.

Baculovirus Scale-Up

Those of you who use CHO PF may have noticed improvements to the technical information regarding cryopreservation provided with your cell line. ECACC and Sigma-Aldrich have been working in partnership to develop better methods for the cryopreservation of cell lines adapted to growth in protein free conditions. We would be interested in hearing from any of our customers regarding their experience of protein free adaptation and cryopresevation. We would welcome the opportunity to share knowledge in this developing area of cell culture.

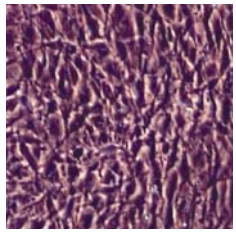
New Cell Lines for the Collection

HCA-7 Colony 29



A human colon carcinoma cell line expressing COX-2. Thank-you to Dr S C Kirkland (Dept. Histopathology, Imperial College, London, UK) for this deposit.

MOCHA



A mouse skin fibroblast with a null mutation in the d (delta) sub-unit of the AP-3 coated vesicle adapter complex. A useful cell line for studying AP-3 assembly and function. Thank-you to Dr. M S Robinson (CIMR, Cambridge, UK) for this deposit.

Do you have any cell lines that you could deposit now?

We are always keen to hear from depositors old and new to have an informal discussion about their cell lines and explain the benefits of depositing with ECACC.

Contact ECACC by email at **ecacc@camr.org.uk** or simply phone +44 (0) 1980 612512 and ask to speak to Joanne Porritt the Senior Scientist within ECACC, responsible for new cell line additions. She will be only too pleased to discuss with you any aspect of depositing cell lines with ECACC.

Information Request 104

Products

Accession No.	Cell Line Name
00102307	CHO PF
02111901	MOCHA
02091238	HCA-7 Colony 29

PRODUCT INFORMATION

Cell Culture Supplements

Cell Culture Tested Antibiotics

There is no substitute for good sterile techniques to prevent microbial contamination. However, if you need to use antibiotics in primary cell culture or for microbial eradication the following table is a guide.

The table below represents a selection from the complete range of Sigma-Aldrich cell culture tested antibiotics, or you can visit our website for further products:
sigma-aldrich.com/antibiotics

Information Request 105

Products

Prod No.	Description	Pack Size	Price £
A2942	Amphotericin B (250µg/ml solution)	20ml 50ml 100ml	6.10 9.20 17.60
A5955	Antibiotic-Antimycotic Solution (100X) Stabilised (10,000 units penicillin, 10mg streptomycin, and 25µg amphotericin B per ml)	20ml 100ml	8.10 21.70
G1272	Gentamicin Sulphate (10mg/ml solution)	10ml 10ml x 10ml	8.00 45.30
G1397	Gentamicin Sulphate (50mg/ml solution)	10ml 10ml x 10ml	31.80 180.60
G9654	Gentamicin-Glutamine Solution (200mM L-glutamine, 5mg gentamicin per ml)	10x5ml	21.80
G1146	L-Glutamine-Penicillin-Streptomycin Solution (200mM L-glutamine, 10,000 units penicillin, and 10mg streptomycin per ml)	10x5ml 100ml	15.70 10.40
G6784	L-Glutamine-Penicillin-Streptomycin Solution Stabilised (200mM L-glutamine, 10,000 units penicillin, and 10mg streptomycin per ml)	10x5ml 100ml	20.90 17.70
K0129	Kanamycin Sulphate (10 mg/ml solution)	20ml	8.00
K0254	Kanamycin Sulphate (50mg/ml solution)	20ml	28.20
N1142	Neomycin Sulphate (10mg/ml solution)	20ml	4.50
N1638	Nystatin Suspension (10,000 units nystatin per ml)	20ml 100ml	11.20 39.90
P4458	Penicillin-Streptomycin Solution Stabilised (50x) (5,000 units penicillin-G and 5mg streptomycin per ml)	20ml 100ml	2.10 4.10
P0781	Penicillin-Streptomycin Solution (100x) (10,000 units penicillin-G and 10mg streptomycin per ml)	20ml 100ml	3.10 7.40
P4333	Penicillin-Streptomycin Stabilised Solution (100x) (10,000 units penicillin-G and 10mg streptomycin per ml)	20ml 100ml	3.80 8.20
P4083	Penicillin-Streptomycin-Neomycin Solution Stabilised (100x) (5,000 units penicillin-G, 5mg streptomycin, and 10mg neomycin per ml)	20ml 100ml	4.90 13.60
T3397	Tylosin Tartrate (8mg/ml solution)	20ml	7.60
P0906	Penicilin-Streptomycin Solution	20ml 100ml	1.70 3.50

Cytokines and Growth Factors

You can now find almost 800 cytokines and growth factors, together with related antibodies and receptors listed in the new Sigma-Aldrich Cell Signaling catalogue. If you have not thought of Sigma-Aldrich as a supplier of these reagents we have listed some of the popular growth factors used by most laboratories. To see the full listing and what's new, go to our website:
sigma-aldrich.com/cellsignaling

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Products

Prod No.	Description	Pack Size	Price £
E2759	Endothelial Cell Growth Supplement; from bovine neural tissue	15mg 5x15mg	61.60 249.10
E0760	Endothelial Cell Growth Supplement; from bovine pituitary	15mg	75.10
E4127	Epidermal Growth Factor; from murine submaxillary gland	5x100µg	470.10
E9644	Epidermal Growth Factor human; rec. expressed in *E. coli*	200µg	118.10
F0291	Fibroblast Growth Factor-Basic human; rec. expressed in *E. coli*	25µg	201.10
G5035	Granulocyte-Macrophage Colony-Stimulating Factor human; rec. expressed in *E. coli*	5µg	165.10
I3769	Insulin-Like Growth Factor-I human; rec. expressed in *E. coli*	50µg	120.10
I9401	Interleukin-1b, human; rec. expressed in *E. coli*	5µg	180.10
T3267	Interleukin-2 human; rec. expressed in *E. coli*	10ku	135.10
I1645	Interleukin-8 human; rec. expressed in *E. coli*	10µg	100.10
L5283	Leukemia Inhibitory Factor human; rec. expressed in *E. coli*	10µg	187.80
N0513	Nerve Growth Factor-7S from murine submaxillary gland	100µg	130.80
N6009	Nerve Growth Factor-2.5S from murine submaxillary gland	10µg	95.60
P4306	Platelet Derived Growth Factor-BB human; rec. expressed in *E. coli*	10µg	175.10
T7039	Transforming Growth Factor-b1 human; rec. expressed in mammalian cells	2µg	175.10
T2815	Transforming Growth Factor-b2 human; rec. expressed in NS0 cells	2µg	235.10
T0157	Tumor Necrosis Factor-a human; rec. expressed in yeast	10µg	165.10
T6674	Tumor Necrosis Factor-a human; rec. expressed in *E. coli*	10µg	159.10
V7259	Vascular Endothelial Growth Factor human;	10µg	200.10

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Media for Biotechnology

CHO Media

We offer three ways to maximize your CHO cell growth and productivity.

Product Code C 5467 (CHO-PF-AF)

Are you looking for maximum productivity in suspension cultures? You've just found it. CHO-PF-AF can support viable cell densities up to 8 x 10^6 cells/ml in batch culture systems, increasing recombinant protein productivity on a per-cell basis by 500% or more.

Product Code C 4726

If you're looking for excellent cell growth and productivity without the presence of hydrolysates or other undefined materials, look no further. This medium is designed to meet the needs of manufacturers seeking optimised downstream processing as well as researchers studying nutritional controls of cellular processes.

Product Code C 8730

Looking for an animal component-free medium to grow CHO cells under adherent conditions. This medium allows cells to remain attached for extended periods and promotes excellent recombinant protein productivity.

Information Request 107

Products

Prod No.	Description	Pack Size	Price £
C5467	CHO Protein-free, Animal Component-free Medium	1L	50.50
		6x1L	224.10
C4726	CHO Chemical defined, Animal Component--free Medium	500mL	23.10
		1L	42.10
		6x1L	213.90
C8730	CHO Protein-free, Animal Component--free Medium for Attached Cells	1L	47.30
		6x1L	212.10

Stemline™ Haematopoietic Stem Cell Expansion Medium

Under recommended conditions, Stemline™ works extremely well for the expansion of progenitors from cord blood, peripheral blood and bone marrow.

Information Request 108

Products

Prod No.	Description	Pack Size	Price £
S0189	Stemline ™ Haematopoietic Stem Cell Expansion Medium	500mL	91.10
		6x500mL	490.80

Animal-Component Free Gene Therapy Medium

Designed for use with the Per.C6® and related cell lines for production of adenoviral vectors, our new medium achieves maximum cell densities and virus productivity without the use of ANY animal- or human-derived components.

Information Request 109

Products

Prod No.	Description	Pack Size	Price £
G9916	Gene Therapy Medium-3 for Adenovirus Production	500mL	26.70
		1L	52.10
G0916	Gene Therapy Medium-1 for Retinoblastoma-Like Cells	500mL	19.00
		1L	39.00
G0791	Gene Therapy Medium-2 for HEK-293	500mL	26.80
		1L	49.70

Hybridoma Media

Only Sigma-Aldrich media can achieve maximum hybridoma cell growth and production without the presence of serum or animal-derived components. Our cGMP-manufactured Hybridoma Media provide:

- Superior cell growth and maximum antibody productivity
- High cell viability for extended periods
- Elimination of risk of contamination from adventitious agents

Information Request 110

Products

Prod No.	Description	Pack Size	Price £
H4409	Hybridoma Medium, Animal Component-free	1L	42.50
		6x1L	190.70
H4281	Hybridoma Medium, Serum-free	1L	36.80
		6x1L	164.60

For a full listing of cell culture media including specialist media visit **sigma-aldrich.com/cellculture**

PRODUCT INFORMATION

Cell Bank Security

How Safe are your Cell Banks?

Ever wondered what would be the consequences of a failure of your own cryostorage facility?

ECACC has for many years provided both the commercial and academic scientific community with a Safe Deposit service facility.

This facility enables organisations to store duplicate stocks of critical cell banks off-site, providing an invaluable second storage. This service is already used by more than 100 organisations within Europe including many large biopharmaceutical companies. The service offered is fully confidential and deposits are only added to the ECACC catalogue or released on receipt of a signed request on company headed paper.
Full details of the terms and conditions upon which the safe deposit service operates can be found on our website **www.ecacc.org.uk**

Authenticated Cell Lines in a Temperature Controlled Liquid Nitrogen Tank

ECACC's standard Safe Deposit Service offers the following benefits:

- Access to safe deposit storage tanks is restricted and logged
- Temperature is maintained below -135°C, constantly monitored and logged with deviations being recorded and reported back to the client
- Regular viability checks carried out on monitor cell lines from safe deposit tanks
- Annual report confirming number of ampoules stored, and integrity of storage conditions including viability results of the monitor cell banks
- Regular internal auditing

A dedicated service is provided for the storage of "GMP" master cell banks. In addition to the benefits afforded by the standard service above this service provides the following additional features:

- Use of a fully validated storage tank
- Only certified fully QC tested (mycoplasma, bacteria, fungi and adventitious viruses) cell banks stored in the tank
- Storage in vapour phase nitrogen only
- Complete transaction logs to record all stock movements
- All stock movements witnessed by QC representatives

Expanding ECACC's Storage Capacity

The recent commissioning of ECACC's new state of the art ultra low temperature storage centre has vastly enhanced both the above services. Secure off-site storage enables ECACC to expand it's storage capacity to accommodate the dramatic increase in the number of cell lines being generated for genetic disease research projects such as those funded by the Medical Research Council (UK) and Wellcome Trust (UK).
We predict that it will add to the current inventory at a rate of 75,000 cryovials per year for at least the next five years. Therefore our aim is to increase the existing capacity by at least 60% to accommodate this demand.

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Cell Bank Security

The New Cell Storage Centre

The new facility will comfortably accommodate 40 large liquid nitrogen vessels with a total capacity of one million cryovials making it one of the largest cell storage facilities of it's kind in Europe. There is the provision to easily expand this in the future to accommodate a further 20 vessels.

Features of this new store include:

- 20,000L liquid nitrogen dual reservoir system
- Automatic fill system for up to 40 storage vessels
- Latest telemetry alarm and temperature monitoring systems using calibrated temperature probes
- Dedicated team permanently based in the facility
- Latest oxygen monitoring systems linked to forced air ventilation
- Electronic inventory control

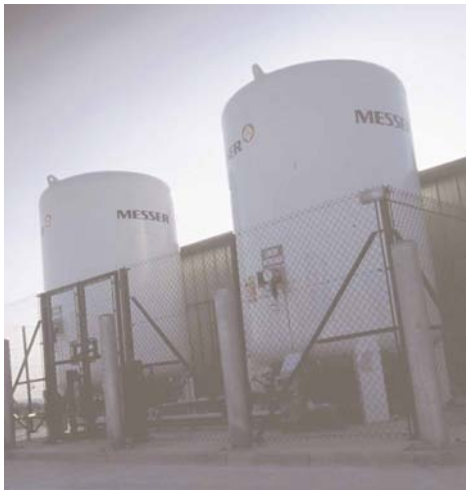
In addition, ECACC has a second storage facility which will enable storage of back-up stocks independant from the new cell store. As further security this site has a completely separate monitoring system and liquid nitrogen is supplied by a second, independent contractor.

How to Use the Service

Prior to sending any samples to ECACC we suggest that you visit our website where you will find a full guide on the procedures and forms which need to be completed before sending your cell banks to us. It is particularly important to note that ECACC must receive a completed biohazard assessment before it can accept any deposits. If you have queries concerning the service itself, or procedures to follow, contact ECACC's technical support direct by email at **ecacc.technical@camr.org.uk or tel +44 (0) 1980 612684.**

The New Cell Storage Centre at ECACC

Facility to hold over a Million Authenticated Cell Lines



20,000L Liquid Nitrogen Dual Reservoir System

European Distribution

Contact and Ordering Information for Cell Culture Products

Austria
SIGMA-ALDRICH HANDELS GmbH
Favoritner Gewerbering 10
1100 Wien
Tel: 43 1 605 8110
Fax: 43 1 605 8120

Belgium
SIGMA-ALDRICH NV/SA.
K. Cardijnplein 8
B-2880 Bornem
Free Tel: 0800-14747
Free Fax: 0800-14745
Tel: 03 899 13 01
Fax: 03 899 13 11

Czech Republic
SIGMA-ALDRICH spol. s r.o.
Pobrezni 46
186 21 Praha 8
Tel: 246 003 251
Fax: 246 003 290

Denmark
SIGMA-ALDRICH DENMARK A/S
Vejlegaardsvej 65B
DK-2665 Vallensbaek Strand
Tel: 43565910
Fax: 43565905

Finland
SIGMA-ALDRICH FINLAND
Teerisuonkuja 4
00700 Helsinki
Tel: (09) 3509250
Fax: (09) 35092555

France
SIGMA-ALDRICH CHIMIE S.à.r.l.
L'isle d'Abeau Chesnes b.p. 701
38297 Saint Quentin Fallavier
Cedex
Tél Numéro Vert: 0800 21 14 08
Fax Numéro Vert: 0800 03 10 52

Germany
SIGMA-ALDRICH CHEMIE GmbH
Eschenstr. 5, 82024 Taufkirchen
Free Tel: 0800/51 55 000
Free Fax: 0800/64 90 000

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SIGMA-ALDRICH (O.M.) LTD
72, Argonafton Str.
16346 Athens
Tel: 0109948010
Fax: 0109943831

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1399 Budapest
Pf. 701/400
Magyarország
Tel: (06-1) 269-6474

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Airton Road
Tallaght
Dublin 24
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20151 Milano
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Fax: 078-6205421

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1081 Oslo
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Fax: 23176010

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Fax: 020-352522
Tel: 08-7424200
Fax: 08-7424243

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Fax: 081 755 28 40

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