SUMMER 2005

Cell Culture

Bringing you the latest information and new products from

ECACC and Sigma-Aldrich

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ecacc SIGMA

Working in Partnership

www.ecacc.org.uk

www.sigma-aldrich.com/cellculture

A Combined Force In Cell Culture

Sensational Prices!

on purchases of classical medium and associated cell culture reagents

When you purchase a cell line from ECACC, it ships with a FREE 500ml bottle of the appropriate 'Classical' medium. This is the exact same medium that ECACC has used to maintain the cell line in culture before freezing or shipping as a growing culture.

We would like to offer you the opportunity to purchase additional media and reagents at truly sensational prices.

To take advantage of this offer, please place your order quoting promotional code X73.

Offer is valid until August 31st, 2005.

Prod No.	Description	Pack Size	Special Price £	€	CHF
B1522	Basal Medium Eagle (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
D1408	Dulbecco's Phosphate Buffered Saline (10x), w/o CaCl and $MgCl_2$	6X500ml	13.00	20.00	30.00
D5671	Dulbecco's Modified Eagle's Medium (1x), w/o L-Glutamine, with 4500mg/L Glucose	6X500ml	13.00	20.00	30.00
D5796	Dulbecco's Modified Eagle's Medium (1x), with L-Glutamine, with 4500mg/L Glucose	6X500ml	13.00	20.00	30.00
D6046	Dulbecco's Modified Eagle's Medium (1x), with L-Glutamine, with 1000mg/L Glucose	6X500ml	13.00	20.00	30.00
D6421	DME/F-12 (1:1) mixture (1x), w/o L-Glutamine, with 15mM HEPES	6X500ml	13.00	20.00	30.00
D6546	Dulbecco's Modified Eagle's Medium (1x), w/o L-Glutamine, with 4500mg/L Glucose, with 110mg/L Sodium Pyruvate	6X500ml	13.00	20.00	30.00
D8537	Dulbecco's Phosphate Buffered Saline (1x), w/o CaCl and $MgCl_2$	6X500ml	13.00	20.00	30.00
D8662	Dulbecco's Phosphate Buffered Saline (1x), with CaCl and $MgCl_2$	6X500ml	13.00	20.00	30.00
G5154	Glasgow Minimum Essential Medium (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
H9394	Hanks' Balanced Salt Solutions (1x), with Sodium Bicarbonate	6X500ml	13.00	20.00	30.00
I3390	Iscove's Modified Dulbecco's Medium (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
L5520	L-15 Medium Leibovitz (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
M2154	Medium 199 (1x), with Earle's Salts, w/o L-Glutamine	6X500ml	13.00	20.00	30.00
M2279	Minimum Essential Medium Eagle (1x), with Earle's Salts, w/o L-Glutamine	6X500ml	13.00	20.00	30.00
M4566	MEM Alpha Modification (1x), w/o L-Glutamine, w/o ribonucleosides and deoxyribonucleosides	6X500ml	13.00	20.00	30.00
M4655	Minimum Essential Medium Eagle (1x), with Earle's Salts, with L-Glutamine	6X500ml	13.00	20.00	30.00
M8028	MEM Joklik Modification (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
M8403	McCoy's 5A Medium (1x) w/o L-Glutamine	6X500ml	13.00	20.00	30.00
N1140	NCTC 109 Medium (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
N6013	Nutrient Mixture F-10 Ham (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
N4888	Nutrient Mixture F-12 Ham (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
R0883	RPMI-1640 Medium (1x), w/o L-Glutamine	6X500ml	13.00	20.00	30.00
R5886	RPMI-1640 Medium (1x), w/o L-Glutamine, with 25mM HEPES	6X500ml	13.00	20.00	30.00
R8758	RPMI-1640 Medium (1x), with L-Glutamine	6X500ml	13.00	20.00	30.00
A5955	Antibiotic Antimycotic Solution, (100x)	100ml	20.00	29.00	44.80
C5914	Cell Dissociation Solution (1x), Non-enzymatic	100ml	14.90	21.50	33.20
D2650	Dimethyl sulfoxide, Sterile filtered, Hybri-Max® Hybridoma tested	100ml	69.60	100.00	154.50
E8008	EDTA solution, 0.02%	100ml	5.20	7.50	11.60
G1272	Gentamicin solution, 10mg/ml, Sterile	10ml	6.20	9.00	13.90
G7513	L-Glutamine solution, 200mm	100ml	7.60	11.00	17.00
H0887	HEPES solution, 1M, Asceptically filled	100ml	30.60	44.00	68.00
H8889	HISTOPAQUE®-1077, Hybri-Max® Hybridoma tested	100ml	19.50	28.00	43.20
I3146	ITS Liquid Media Supplement, (100x)	5ml	13.60	19.50	30.00
M7145	MEM Non-essential Amino Acid Solution, (100x)	100ml	6.20	9.00	13.90
P0781	Penicillin - Streptomycin Solution (100x), (10,000units/ml Penicillin and 10mg/ml Streptomycin	100ml	8.00	11.50	17.80
P3539	Penicillin - Streptomycin, Powder, cell culture tested	50ml	7.30	10.50	16.20
P4333	Penicillin - Streptomycin Solution (100x), Stabilised	100ml	9.00	13.00	20.00
S8636	Sodium pyruvate solution, 100mm	100ml	4.90	7.00	10.80
S8761	Sodium bicarbonate, 7.5% solution	100ml	4.20	6.00	9.30
T3924	Trypsin-EDTA solution, (1x)	100ml	8.00	11.50	17.80
T4049	Trypsin-EDTA solution, (0.25%)	100ml	7.30	10.50	16.20
T4174	Trypsin-EDTA solution, (10x)	100ml	9.40	13.50	20.90
T8154	Trypan Blue solution, 0.4%	100ml	6.20	9.00	13.90

Dear Cell Culturist

Welcome to the Summer edition of the joint ECACC-Sigma Cell Culture Newsletter. Once again, we have attempted to bring to you a varied range of articles that are interesting, technically informative and have relevance to your work.

The generation of this Newsletter has again highlighted the synergy of the ECACC-Sigma partnership, and more specifically, how this can provide a superior service to you, the cell culturist.

The Sigma-specific articles illustrate the immense depth of our product portfolio, both within cell culture and other associated Life Science disciplines (see Whole Genome Amplification, Pg 21). Our philosophy at Sigma is not to view cell culture as an isolated science, but rather as an integral part of a much larger picture. Here we have highlighted products associated with the increasingly important field of recombinant protein expression; a new chemically-defined medium for culturing CHO cells, a CHO medium Optimisation Kit and an animal component-free insect medium designed for culturing sf9 and sf21 cells. However, Sigma is also able to offer you a comprehensive range of associated products for conducting expression work in these insect cells, and in conjunction with ECACC, the cells themselves! In effect, we have created the complete package, making your work easier and more effective.

Sigma-Aldrich has recently clearly signalled its long-term commitment to the cell culture community. It is now truly a world-leader in cell culture. We are taking an industry leading position; delivering innovation and service to the market, including such critical technologies as animal component-free media and optimisation services, whilst enhancing customer service, excelling in quality, and leading in delivery.

Sigma is setting new standards in the ability to work with its customers from research, through development to commercialisation. From microtitre plate to Bioreactor, cutting time and development costs.

And, through our Partnership with ECACC, Sigma is not only able to supply you with your cell culture consumables, but also the cells necessary to conduct your research.

From ECACC there is an in-depth article which discusses the rise in significance of its Human Genetic Services highlighting how it has developed to meet the needs of the genetic research community, particularly the provision of DNA and RNA from large cohorts. ECACC is also proud to be launching its brand new online interactive forum, CultureTech, where scientists around the world can exchange views and comments on current developments in cell culture. As many of you are already aware, ECACC has launched a new 300 page hard copy catalogue which lists the full range of products and services offered including detailed listings of its General Collection cell lines.

We trust you will find this newsletter of interest. However, feedback from our customers is always welcomed so please do let us have your views on what you would like to see in future editions. Don't forget, to continue to receive this newsletter in the post, you need to register by completing the reply card.

Yours sincerely,

Simon Kucia
European Sales Development Manager Cell Culture Sigma-Aldrich

Bryan Bolton
Sales & Marketing Manager European Collection of Cell Cultures

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Register for future editions of the Cell Culture Newsletter

To ensure that you receive future editions of the ECACC-Sigma Cell Culture Newsletter, please can we ask you to fill out the **Business Reply Card** and return it to us as soon as possible.

Thank you.

A Limitless Source of Genomic DNA or RNA

Studying a Cohort or a Few Individuals? – Would You Benefit From a Reproducible and Renewable Supply of Each Patient's Genomic DNA or RNA?

Isobel Atkin

European Collection of Cell Cultures, Health Protection Agency, Porton Down, Salisbury, Wiltshire, SP4 0JG, U.K.

The ultimate aim of the generation of a lymphoblastoid cell line from a patient's blood sample is to secure a renewable supply of that individual's genomic DNA. A cell line can be cryopreserved and resuscitated for cultivation and subsequent DNA and/or RNA extraction at any time. The Human Genetic Cell Bank (HGCB) Service enables clinical research groups to secure valuable case and control genomic material for genetic analyses. This removes the need to return to individuals for a second blood sample and the risk of exhausting unique DNA stocks.

Renewable Source

The Human Genetic Cell Bank Service is based on the preparation and Epstein-Barr Virus (EBV) immortalisation (also known as transformation) of human peripheral blood lymphocytes (PBLs) resulting in the production of lymphoblastoid cell lines (LCLs). These cell lines are subsequently banked, providing a renewable and expandable source of genomic DNA. Please see Figure 1 for an overview of the process.

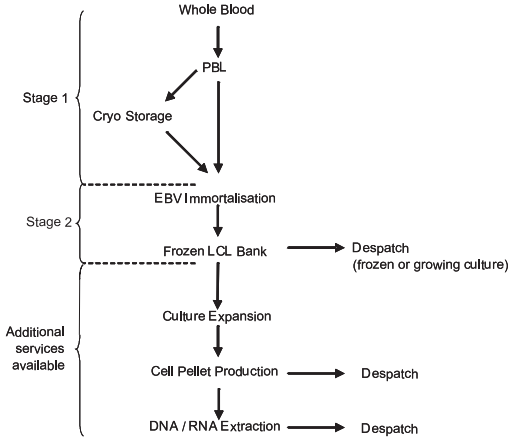

Figure 1. The main elements of the EBV transformation service

Application

With advances in molecular biology, a number of methods for whole genome amplification have become available and evidence is accumulating that it can give good results in genotyping (high call rates and concordance). This is considered a good method of stock replenishment where there is no alternative. However, whole genome amplified DNA cannot be used either in the study of DNA methylation or of very large genomic fragments (pulsed field gel electrophoresis). Moreover, it has not been validated for studies in gene amplification or repetitive gene sequences. It may also be unsuitable for study of telomeric repeats. By contrast, DNA from LCLs is suitable for all such studies. Furthermore, LCLs are suitable for studies on both transcriptional and translational products. ECACC is experiencing increasing demand for RNA extracted from LCLs from individuals with a range of genetic diseases such as asthma, diabetes and dementia.

Figure 2. Blood spot cards

Quality & Expertise

The HGCB has provided an EBV lymphocyte immortalisation service to human genetic research in the UK since 1986 and has amassed samples representing over 100,000 donor subjects. The HGCB currently initiates more than 1000 EBV transformations per month, the majority of which use PBLs resuscitated from liquid nitrogen storage. Normally ECACC achieves an average transformation success rate of >95% at first attempt. All procedures are managed in accordance with the quality standard BS:EN:ISO9001:2000. Quality control measures in place include mycoplasma screening and determination of cell numbers, percentage viability and sterility. Blood samples are retained on blood spot cards (see Figure 2) so that the identity of any derived lymphoblastoid cell line can be verified by comparison with the source material using DNA profiling techniques.

Current Users of the Service

The HGCB service has a diverse range of customers from researchers that deposit just a few blood samples a year from patients with rare genetic disorders to large studies submitting samples from thousands of individuals. These large studies have included Medical Research Council (MRC) funded investigations into the genetics of diseases such as Alzheimer's, Type 2 Diabetes, Multiple Sclerosis and Hypertension (see Table 1). The HGCB Service has been responsible for generating LCLs for two large control cohort collections – the MRC National Survey of Health and Development (NSHD) '1946 Cohort' and the National Child Development Study '1958 Cohort' in close collaboration with the Avon Longitudinal Study of Parents and Children (ALSPAC) for which the Wellcome Trust has provided funding. In addition, the HGCB has produced the cell lines from which human random control (HRC) genomic DNA is extracted for the HRC genomic DNA panels available from ECACC.

Developing the Service

At ECACC we are continuously seeking to improve all aspects of our service. Jim Cooper leads a Development Team which works alongside the HGCB and is dedicated to improving the existing HGCB service and developing new systems. 'We have a system that works well. Our aim is to further maximise the efficiency and capability while reducing costs using techniques such as miniaturisation and robotics. We are also investigating new technologies', explains Jim.

ECACC was awarded a Medical Research Council (MRC) grant in November 2002 for the development of the HGCB service as part of the MRC national DNA banking initiative. The key aims of this project are to improve the throughput of the system, identify ways to reduce costs and define alternative protocols for the transformation of PBLs.

A multiwell test platform has been generated for process development and reagent qualification which allows key parameters to be tested in a tightly controlled model. All steps of the process have been studied including: media volumes, cell concentration, EBV multiplicity of infection and serum concentration.

A key achievement of the project has been to secure a source of blood samples from the National Blood Service representing a "normal" population. This valuable resource allows new technologies to be tested using a standard set of samples, enabling direct comparison of results.

In general, approximately 2-7% of samples do not transform at the first attempt. There is a widespread belief that cells from certain individuals are resistant to EBV transformation. However, investigations suggest that failed transformation is most commonly due to poor blood sample quality - principally low numbers of viable PBLs. ECACC scientists are developing ways to assess the quality of samples according to defined criteria and so adjust protocols to enhance the potential for transformation success.

Alternative protocols are also being examined and developed. Significant steps have been made towards developing a protocol for whole blood transformation. This removes the need to separate peripheral blood lymphocytes prior to transformation, so reducing the number of process steps required.

Keeping the Service on Track

The early introduction of an electronic barcode-driven tracking system at the HGCB has had a major impact on sample throughput. The purpose built Laboratory Information Management System (LIMS) allows a paperless laboratory.

Study	Depositor	No. of samples
Alzheimer's Disease (late onset)	Prof. J Williams, University of Wales, College of Medicine, Cardiff	3500
Type 2 Diabetes Family	Prof. A Hattersley, Peninsula Medical School, Exeter	4000
Asthma/Eczema	Prof. W Cookson. Wellcome Trust Centre for Human Genetics, Oxford	2000
Multiple Sclerosis	Prof. A Compston, Addenbrookes Hospital, Cambridge	2000
Hypertension (BRIGHT)	Prof. M Caulfield, William Harvey Research Institute, London	4300
Unipolar Depression	Prof. P McGuffin, Institute of Psychiatry, London	1500
Family Heart Disease	Prof. A Hall, University of Leeds	4800
Glomerulonephritis	Prof. A Rees, University of Aberdeen	2200

Table 1. Medical Research Council DNA banking projects which have submitted blood samples and peripheral blood lymphocytes to the HGCB

Figure 3. Barcode-driven Tracking System

Hand-held, programmable, wireless scanners enable samples to be tracked and identified anywhere in the process (see Figure 3). A full audit trail history can be rapidly generated. A significant factor for the successful development of this tailor-made system is the dedicated IT personnel employed at ECACC. In addition to LIMS, the ECACC IT department has also developed 'ICAST' a robust electronic inventory control system.

Supply of DNA & RNA

The Wellcome Trust has supplied grant funding to ECACC, from autumn 2004, in order to expand the HGCB service to accommodate Wellcome Trust funded projects. This grant has enabled ECACC to invest in robotic technologies for the extraction, quantification, normalisation and generation of aliquots of DNA.

Advancements in the extraction of DNA and RNA from lymphoblastoid cell lines are on-going at ECACC. The Cell Products Team routinely generate very pure, high molecular weight genomic DNA suitable for a wide spectrum of genetic research applications. This will be expanded to include RNA in the future.

The human random control (HRC) genomic DNA panels, produced by the Cell Products Team, represent DNA from a control population of 480 individuals and are available for immediate use. In addition to DNA derived from LCLs, ECACC has also developed a process for the removal of white blood cells from leucocyte reduction filters for subsequent DNA extraction. Extraction of DNA from blood filters can enable very large control populations to be generated; potentially in excess of 1000 random control DNA samples can be produced upon request.

Many researchers appreciate the convenience of ordering DNA ready prepared from their cell lines of interest so that they are not required to maintain cell cultures themselves. Researchers need not be burdened with the recruitment and generation of control material. Rather they can purchase control DNA from ECACC and focus on the assays they wish to perform such as genotyping, linkage mapping and SNP analysis.

For information on using the HGCB service and/or the DNA/RNA products available from ECACC please consult the ECACC website **www.ecacc.org.uk** or contact us on **+44 (0) 1980 612512 or email: ecacc.technical@hpa.org.uk**

Have You Considered Depositing Cell Lines with ECACC?

Isobel Atkin and Peter Thraves
European Collection of Cell Cultures, Health Protection Agency, Porton Down, Salisbury, Wiltshire, SP4 0JG, U.K.

- **Do You Have a Valuable Cell Line?**
- **Are You Equipped to Supply Your Cell Line on Demand?**
- **Do You Want to Avoid Depletion of Stocks of Cell Lines You Have Generated?**
- **Would You Like Greater Awareness of Your Cell Line?**

Establishing a new cell line requires much time and energy. Once established and characterised, cell cultures are valuable research tools and like any other valuable resource they need to be secured and protected to ensure their future availability and integrity. In addition, the distribution of cell lines to colleagues, collaborators and other interested parties can be a burden. In light of this, for more than two decades, researchers have opted to deposit cell lines with ECACC.

Depositing cell lines into the ECACC collection is free of charge, straightforward and offers many benefits. Laboratories that do not have a service function can find handling requests for cell lines an administrative, financial and time-consuming drain. We provide a cell banking service to the scientific community and have the expertise and facilities required for the maintenance, storage and distribution of important cultures.

Strictly controlled banking procedures result in the generation of Master and Working cell banks of deposited cell lines. The banks are stored in ECACC's state-of-the-art liquid nitrogen repository with multiple levels of security and protection. If a depositor's own cell line stocks are compromised they are safe in the knowledge that a bank is secured at the ECACC facility.

Prior to being made available for distribution all cell lines deposited into the collection undergo thorough authentication and quality control tests free of charge. DNA profiles are generated to enable identification of the cell line and tests for microbial contamination, including mycoplasma, are performed. In this way researchers are able to ensure collaborators and colleagues have access to viable, authenticated and quality controlled stocks.

Dr. Alan Morris, a Reader in the Division of Clinical Sciences at the University of Warwick Medical School, has deposited several human oesophageal cell lines with ECACC. These cell lines have consistently increased in popularity. Interest in the cell line OE33 (Figure 1), originally isolated from an adenocarcinoma of the lower oesophagus, has been particularly strong since year 2001. Dr. Morris cites the principal reasons for depositing with ECACC were for security of the cell lines and to benefit from the distribution and publicity offered. It has enabled him to direct interested parties to ECACC confident in the knowledge that they will receive and begin their work with authenticated and qualified stocks of the cell lines. The costs and responsibility of administering to the distribution of the cell line around the world are removed. Dr. Morris added that he would definitely consider depositing cell lines into the ECACC collection in future.

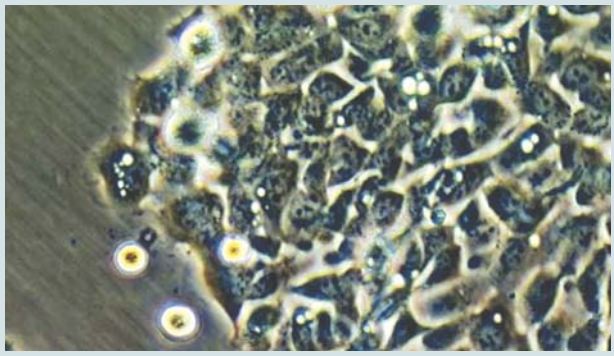

Figure 1. Human Oesophageal Carcinoma (OE33)

All relevant data associated with a deposited cell line is collated and published on the ECACC website and in the hardcopy catalogue. This results in international promotion of the cell line and increases recognition of the depositor's research.

NEW Deposits

Vero/hSLAM
Catalogue no. 04091501

Vero/hSLAM is a derivative of the African green monkey kidney cell line Vero that has been genetically modified to express human signalling lymphocytic activation molecule (SLAM), also known as CDw150, which is a receptor for measles virus. These cells can be used to isolate measles virus from human clinical samples and are a substitute for the B95a cell line for this purpose. Mumps susceptibility has also been demonstrated at the depositor's laboratory. This cell line was deposited by Dr. Bernard Cohen of the Virus Reference Department, Centre for Infections, Health Protection Agency, UK.

L929/R
Catalogue no. 04102001

L929/R is a cisplatin-resistant cell line developed by exposure of the parent L929 murine fibroblast cell line to increasing concentrations of cisplatin *in vitro*. L929/R cells can be used in the development of novel anti-cancer treatments. The parent cell line L929 was derived from normal subcutaneous areolar adipose tissue. This cell line was deposited by Dr. Stephen Merry of the Faculty of Health Sciences, Staffordshire University, UK

Positive sentiments have been expressed by Dr. Tim Ward a clinical scientist at the Paterson Institute for Cancer Research, Manchester. Dr. Ward is involved in anti-cancer drug development and through his research has generated a variety of drug resistant cell lines. He has deposited a selection of cell lines with ECACC. This includes cell line A2780cis (Figure 2) derived following exposure of the human ovarian cancer cell line A2780 to increasing concentrations of cisplatin, an anti-cancer agent.

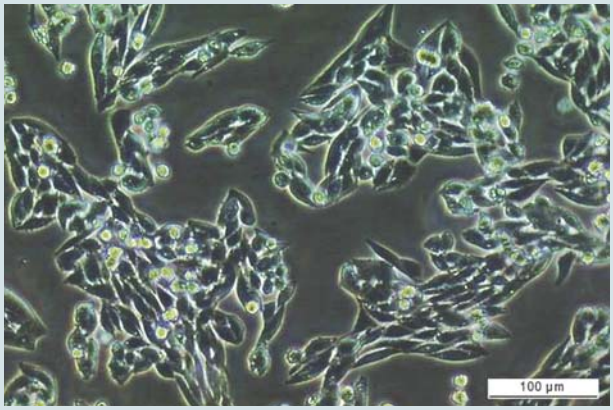

Figure 2. Human Ovarian Carcinoma (A2780cis)

'Generating drug resistant cell lines takes a lot of time, depositing these into the ECACC catalogue offers us security', says Dr. Ward. He appreciates that depositing with ECACC allows staff to focus on their research activities rather than being tied up with satisfying requests for their cell lines. 'When requests are received for the cell lines our stocks are not depleted', added Dr. Ward.

A2780cis has been very useful for studying drug resistance and can be used for screening novel compounds for known drug resistance. Dr. Ward emphasised the importance of depositing the parental cell line A2780 in conjunction with A2780cis. 'Although A2780 could be obtained from other sources it was essential to deposit the original parental cell line from which the drug resistant cell line was derived. The parental cell line being genetically close to the drug resistant cell line provides the most appropriate control.' In addition to A2780cis, Dr. Ward has also deposited A2780adr, an adriamycin resistant cell line also derived from A2780. This group of cell lines is one of a number of sets of drug resistant cell lines maintained by ECACC.

Depositors are reassured that ECACC does not claim ownership of cell lines placed in the collection but serves as custodian. In some cases depositors may prefer to restrict the distribution of their cell lines and specify that release forms are completed before distribution. ECACC is very flexible in imposing release conditions on cell line deposits should a depositor wish to have more control on who receives their cell lines. Alternatively, in the case of cell lines with potential commercial value, ECACC can establish a framework for securing and licensing the property to create revenues for the inventor/donating organisation. Requests for the commercial use of a cell line will always be referred back to the depositor.

We are constantly striving to improve the range of cell lines available from our cell collections in order to reflect the needs of current life science research. Recent deposits include a series of lentiviral packaging cell lines and a monkey cell line stably expressing a human measles virus receptor. If you have generated a new cell line, whether it is derived from a tumour biopsy, a drug resistant variant or a genetically modified clone, why not consider depositing with ECACC? We aim to ensure that the deposit of cell cultures into our collections is a trouble-free experience.

For more information and advice please contact The Head of General Collection at ECACC, Dr. Peter Thraves on **tel: +44 (0)1980 612512, email: ecacc.technical@hpa.org.uk**

Cell Line	Cat No.	Description	Exclusive to ECACC
A2780	93112519	Human ovarian cancer cell line	✔
A2780ADR	93112520	Adriamycin-resistant derivative	✔
A2780cis	93112517	Cisplatin-resistant derivative	✔
COR-L23	92031919	Human Caucasian lung large cell carcinoma	✔
COR-L23/CPR	96042336	Cisplatin-resistant derivative	✔
COR-L23/5010	96042338	Doxorubicin-resistant derivative	✔
COR-L23/R	96042339	Doxorubicin-resistant derivative	✔
COR-L23/R23	96042337	Doxorubicin-sensitive revertant	✔
H69	91091802	Human Caucasian lung small cell carcinoma	✘
NCI-H69/CPR	96042328	Cisplatin-resistant derivative	✔
NCI-H69/LX10	96042331	Doxorubicin-resistant derivative	✔
NCI-H69/LX20	96042332	Doxorubicin-resistant derivative	✔
NCI-H69/LX4	96042329	Doxorubicin-resistant	✔
NCI-H69VCR/R	96042330	Vincristine resistant derivative	✔
MOR/0.2R	96042335	Human lung adenocarcinoma Doxorubicin-resistant derivative	✔
MOR/0.4R	96042334	Doxorubicin-resistant derivative	✔
MOR/CPR	96042333	Cisplatin-resistant derivative	✔
K562	89121407	Human Caucasian myelogenous leukaemia	✘
K562 cl.6	85011407	A subclone of the parent line K562	✔
K562 AZQR	93112521	Benzoquinone-resistant derivative	✔
EMT6/AR1	96042327	Mouse mammary tumour cell line Doxorubicin-resistant derivative	✔
EMT6/AR10.0	96042326	Doxorubicin-resistant derivative	✔
EMT6/CPR	96042318	Cisplatin-resistant derivative	✔
EMT6/MTXR	96042322	Methotrexate-resistant derivative	✔
EMT6/VCR/R	96042323	Vincristine-resistant derivative	✔
EMT6/VRP/R	96042319	Verapamil-resistant derivative	✔

Note: Many of these cell lines exhibit cross-resistance to other drugs.
Doxorubicin is also known as adriamycin

Table 1. Examples of sets of drug resistant cell lines available from ECACC

News from the UK Branch of the ETCS

ETCS Meeting, York, March 21st

The UK branch of the European Tissue Culture Society (ETCS-UK) facilitated a highly successful 2-day symposium at the Central Science Laboratories, York, March 21-22. The symposium topic was "Models of Stem Cell Biology", organised by Dr. Paul Genever and Dr. Claire Varley, University of York, together with Dr. Andy Scutt from the University of Sheffield, who was responsible for the programme. The ECACC provided administrative support and sponsored the abstracts/programme book. Sigma-Aldrich and ECACC exhibited at the meeting.

The programme was divided into four Sessions, each of four presentations, that focused on:

1. Stem Cells: their biology, differentiation and culture requirements.
2. 3-Dimensional Cell Culture: neuronal stem cells, retinal cells and bladder epithelial cells.
3. Tissue-specific Differentiation: chondroprogenitor cells, muscle cells and bladder epithelial cells.
4. Transdifferentiation: stem cells/progenitor cells in the context of liver function, chondrocytes and peritoneal mesothelial cells.

The Sessions were enabled by an impressive group of invited speakers with established reputations in their specialist fields. All were excellent and the standard of science was extremely high. ETCS-UK extends particular thanks to Dr. Steven Minger, who "jetted" in from a previous scientific meeting in the nick of time to deliver his presentation, then immediately "jetted" out to another meeting. Details of the contributors can be found on the ETCS-UK pages of the ECACC website.

Professor Ian Mackenzie (University of London) chaired a thought-provoking workshop on "Cancer Stem Cells", primed by presentations from himself together with Dr. Anne Collins (University of York). The central theme was that only a small proportion of tumour cells are tumourigenic, and that this sub-population has stem cell-like characteristics. These "cancer stem cells" are capable of generating new clones containing additional stem cells as well as regenerating phenotypically mixed populations of non-clonogenic cells present in the original tumour.

The symposium was well attended by approximately 100 delegates, and the organisers were congratulated. ETCS-UK has maintained a long tradition of good quality scientific meetings and the next meeting is being planned. Monitor the ECACC website for further news.

David Lewis
Treasurer, ETCS-UK

Getting Tissue Culture Closer to Life

Terence Partridge
Genethon, 1 bis rue de l'Internationale, BP 60 - 91002 Evry Cedex, France

For the most part, primary tissue culture of any given cell type entails dissociation of the tissue in which that cell naturally resides and plating it out onto a suitable substratum using a medium that is compatible with its growth. Such schedules commonly include a procedure for enriching or ideally for purifying the cell of interest from the mixture of cells released from the original tissue. Skeletal muscle has been an interesting variant on this general approach, since the mature muscle cells are very delicate once removed from their surroundings and do not survive most isolation procedures. Muscle tissue is normally cultured from the precursor cells present in muscle, which are generally thought to be synonymous with the satellite cells that lie sandwiched between the plasmalemma of the muscle fibre and the overlying basement membrane. The subsequent proliferation, differentiation and fusion of the muscle precursors to form multinucleate 'myotubes' has become one of the more popular models of tissue differentiation, being quite reproducible in some hands.

As a model of muscle in the body however, this type of culture has a number of shortcomings. Some of these arise from the failure of the myotubes to progress to the later stages of differentiation probably reflecting our inability to properly reproduce the normal *in vivo* environment in which the muscle fibres are innervated, vascularized and linked via tendons to a compliant skeletal system on which, their normal function would require them to exert tension. Other deficiencies are to do with the uncertainty of provenance of cells isolated from muscle by standard enzymatic disaggregation techniques.

This uncertainty raises 3 types of question. Are all of the myogenic cells derived from satellite cells? Are all satellite cells myogenic? How representative are the cells in culture of the myogenic population responsible for myogenesis *in vivo*? These can all be answered, in large part, by using muscle fibres isolated cleanly from the muscle by digestion with carefully selected batches of collagenase as starting material for culture [1]. If such preparations are made cleanly, using only fibres that are not hyper-contracted as starting material, it is possible to eliminate contamination by non-muscle cells and to be sure that all cultures start only from satellite cells. The fibre, being the standard unit of muscle, forms a basis of comparison for calculating differences in yield between muscles, or animals, or treatments, or other variables. It is also possible to characterize the starting muscle fibres in terms of the numbers of satellite cells they carry, for comparison with how many myogenic cells they produce within a chosen time period [2]. By such means, tissue culture can be used as a monitor of the myogenic qualities of the muscle from which they were derived and to make quantitative inferences that relate directly to myogenesis *in vivo*. A further aspect of this type of preparation, is that the freshly prepared fibre provides a highly synchronised model of activation of a quiescent precursor into a pathway involving both self replacement and production of differentiated tissue [3].

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A Novel Process for Cell Microencapsulation

'Tandem' Hydrogels

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Introduction

The ability to introduce cultured cells into a biological system without concerns for adverse biological reactions or mechanical damage offers a number of potential medical, technical and economic benefits. The protection of "guest" cells from a host immune system (immunoisolation), or from mechanical damage in a bioreactor, can be approached by encapsulating the cellular material within a membrane or a capsular matrix, that allows nutrients, wastes, and therapeutic products to permeate and diffuse freely, while acting as a barrier to external influences such as mediators of a host immune system. We are specifically interested in microencapsulation, where sub-mm spherical, hydrogel-based constructs (capsules if hollow, beads if solid) are used for entrapping individual or aggregated cells. Such systems are commonly used for cell transplantation purposes[1].

Materials for Cell Encapsulation

Calcium alginate has been the most commonly used material for many years. Calcium alginate is a polysaccharide naturally occurring in its sodium salt form. It produces gels through a particularly cell-friendly process, which is based on a calcium-induced ionotropic gelation, a physical process where multivalent cations (such as calcium) bridge and thus physically cross-link polymer chains[2, 3]. This process has the advantage of rapid kinetics: cells can be dispersed in an alginate solution, which immediately solidifies when exposed to a calcium solution. However, alginate suffers some drawbacks. Variability in its chemical structure affects the strength and homogeneity of the gel structure, the reversibility of the gel (kept together by extractable calcium ions), and the frequent presence of endotoxins. Improved purification procedures and polyelectrolyte complexation on the surface of the alginate beads have partially overcome these problems. However, appropriately designed synthetic materials may provide better alternatives.

Chemically cross-linked synthetic hydrogels comprise a type of material that is inherently irreversible and possibly tougher than alginates. Their method of manufacture is also more controllable. A major disadvantage, however, hampers the use of such systems: even if cell-benign reactions are used for cross-linking, their kinetics is generally not compatible with processing techniques that require an 'instantaneous' gelation. Chemical reactions are "slow" compared to purely physical interactions.

Tandem Hydrogels

In the preparation of calcium alginate (and most other physical gels) there is a substantial overlap between morphogenesis of the material (the 'gelation' or sol-gel process) and development of its final mechanical and transport properties. We have realised that these two aspects can be easily decoupled: for example, by combining a quick physical gelation with a slower chemical cross-linking, it is possible to obtain a solid material with the rapid kinetics typical of physical processes. On the other hand, after an appropriate incubation time, superior mechanical properties and irreversibility are achieved by the formation of a network of covalent bonds. We have described the use of these two different hardening processes as tandem gelation because, while each independently provides a result similar to a hydrogel, together they interact synergistically to allow improved performance.

Specifically, we have used synthetic poly(ethylene glycol)-poly(propylene glycol) triblock copolymers, known as Pluronics or Poloxamers when linear, as Tetronics or Poloxamines when 4-armed, which present a reverse thermal gelation in water solution[4 5]: The result is soluble in cold water, and forms gels at a range of physiological temperatures. By introducing an appropriate number of reactive functional groups at the chain termini (thiols and electron-poor carbon-carbon double bonds), such polymers can also undergo an irreversible gelation through the occurrence of a Michael-type addition cross-linking reaction[6, 7] (Figure 1).

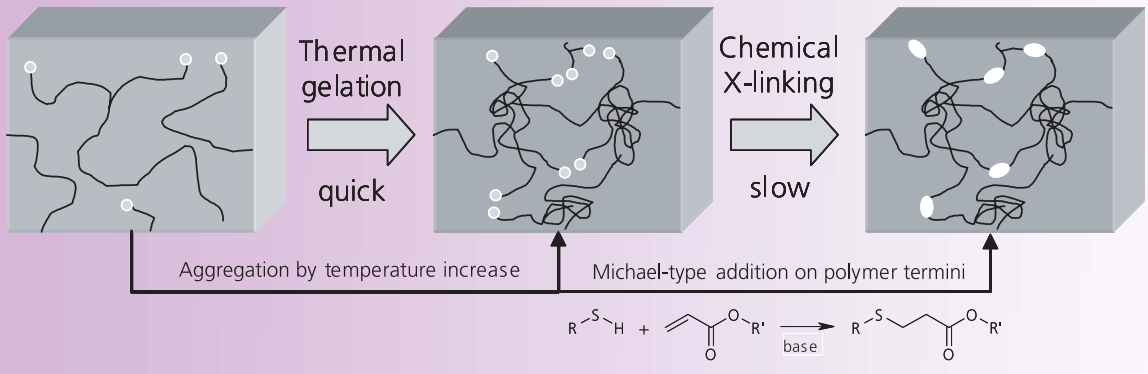

Figure 1. Schematic view of a 'tandem' process. The physical gelation determines a quick hardening process; the material is then cured with a slower kinetics.

Initially, the polymers can be dissolved in water at low temperature (5-10°C) and controlled pH (6.8-7.2). Under such conditions the physical gelation is avoided and the chemical reactions take place at a negligible rate, because Michael-type reaction kinetics is pH-dependent. Upon exposure to a physiological environment (37°C and pH7.4), these solutions rapidly gel and with a slower kinetics chemically cross-link[6, 7].

This 'tandem' gelation mechanism allows to obtain materials resembling alginate in a) hardening kinetics, due to the fast thermal gelation, b) mechanical and transport properties, due to the possibility of adjusting the gel mesh size in the chemical curing, c) biocompatibility, due to the PEG content and the mild conditions of the Michael-type addition, that proceeds smoothly and quantitatively in physiological conditions without generation of toxic by-products or other undesired effects on cell metabolism[7].

The processing conditions have been optimised for the production of microbeads and microcapsules (Figure 2), generated in a commercial jet-breakup encapsulator in order to achieve a tight size distribution. Preliminary experiments for the entrapment of CHO cells have showed that they entirely preserve their viability after encapsulation[8].

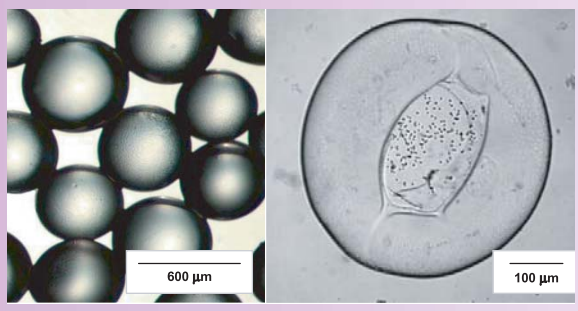

Figure 2. *A.*Collection of regularly shaped Tetronic gel micro-beads (diameter 500÷600µm) photographed after removal of the suspending liquid bath. *B.* Tetronic gel capsule (average shell diameter 900µm) loaded with viable CHO cells. The capsules are produced employing the double nozzle technique so that the gel material is present only in the shell and cells are confined in the liquid core of the particle.

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Join the European Tissue Culture Society

Tissue culture is a research tool that has application across a broad spectrum of scientific disciplines. Rarely, now is it an end in itself and essential cell culture skills are often subverted to the primary research aims. There are numerous examples of research programmes that are impeded or invalidated by poor, basic tissue culture practises.

The European Tissue Culture Society (ETCS) was formed in 1979 to promote the application of tissue culture to problems of cell biology and to provide an across-discipline forum within which scientists who use tissue culture could exchange information and experience. It was, and remains a particular aim of the Society to help young research scientists who need to acquire cell culture skills in order to conduct their research.

Most European countries have a national branch of the ETCS and the network is administered by a Council of members and a central Executive Committee. Details can be found on the main ETCS website **(www.etcs.info)**. Each national branch organises programmes of scientific meetings and every 2 years one member nation will organise an international meeting. All such meetings are publicised on the main website.

The UK branch of the ETCS (ETCS-UK) seeks to prosecute the aims of the ETCS mainly through organising or facilitating a programme of scientific meetings and workshops that focus on topical areas of cell science, or specialised applications of tissue culture. The York meeting (reported above) is an example. ECACC and Sigma support ETCS-UK and ECACC provides secretarial and administrative resources to the Branch. ECACC also dedicates pages of its own website **(www.ecacc.org.uk)** to ETCS-UK news and announcements.

ETCS-UK is run by a committee which is presently comprised of Professor Ian Mackenzie (Chairman), Dr David Lewis (Treasurer), Dr Caroline Wigley and Dr Angela Hague. Professor John Masters is President of the central European Executive Committee and also attends UK Branch Committee meetings.

Membership of ETCS-UK costs £20 per year (£10 to students), which entitles them to discounted attendance at all Society meetings. The discounts more than recoup the cost of membership. Members will receive newsletters both from ETCS-UK and the central organisation, together with early notification of forthcoming meetings. Income from membership fees goes towards administration costs and helps to underwrite the costs of meetings.

To apply for membership of the UK Branch, please email **lisa.reynolds@hpa.org.uk** and include your postal address so that we can send you an application form. To join any other national branch see the central ETCS website.

David Lewis
Treasurer, ETCS-UK

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CHO Kit 2

Medium Optimisation Kit for CHO Cells:

Using Factorial Matrix Design of Experiment to Increase Cell Performance

Brigid DeLong, Nan Lin, Scott Ross, and Matthew Caple

Sigma-Aldrich Corporation, St. Louis, MO, USA

- **Statistical approach to medium development using Design of Experiment software**
- **Animal component-free, with chemically defined formulation options**
- **Efficient medium optimisation of any CHO clone**

Introduction

Chinese Hamster Ovary (CHO) cells are of great interest for bioprocessing and pharmaceutical research and development. These cells are robust in culture and are able to produce a variety of recombinant glycoproteins at high levels on a large scale. The most challenging aspect of culturing recombinant CHO cell clones is providing for the diverse nutritional requirements that are unique to every transfected cell line – often requiring the development of a custom medium for each particular clone. The traditional approach to media development involves titrating each component individually to determine the optimal level of supplementation. This process involves extensive testing conditions and is very lengthy. Therefore, using factorial matrix statistical assays to accelerate the optimisation of cell culture medium has received great attention in many pharmaceutical companies.

Design of Experiment (DOE) Provides Efficient Experimental Procedures and Easy Analysis

In order to assist pharmaceutical companies in improving their medium optimisation process, Sigma-Aldrich has developed CHO Kit 2 (Prod. No. C4364) – a medium component optimisation kit that utilises DOE. The design of this kit allows the researcher to test several supplements simultaneously at high and low levels, with all possible combinations based on factorial matrix design. This allows the researcher to recognise interactions between components in fewer conditions than the traditional approach without losing important information. This novel approach greatly reduces the time and effort needed to optimise a medium for a particular CHO clone. Figure 1 outlines the procedure recommended for the testing of the kit supplements.

The statistical analysis software, Design Expert®, is used with CHO Kit 2 to analyse the factorial matrices. In brief, the statistical programme calculates the effect of any supplement or group of supplements against variance in the assay based on the data collected using mathematical modeling and prediction. The data can be collected and analysed based on different optimisation endpoints, such as maximum cell density, integrated cell area (cell days), or recombinant protein production. Design Expert® analysis can predict an infinite number of combinations of the supplements to design solutions specific to your needs.

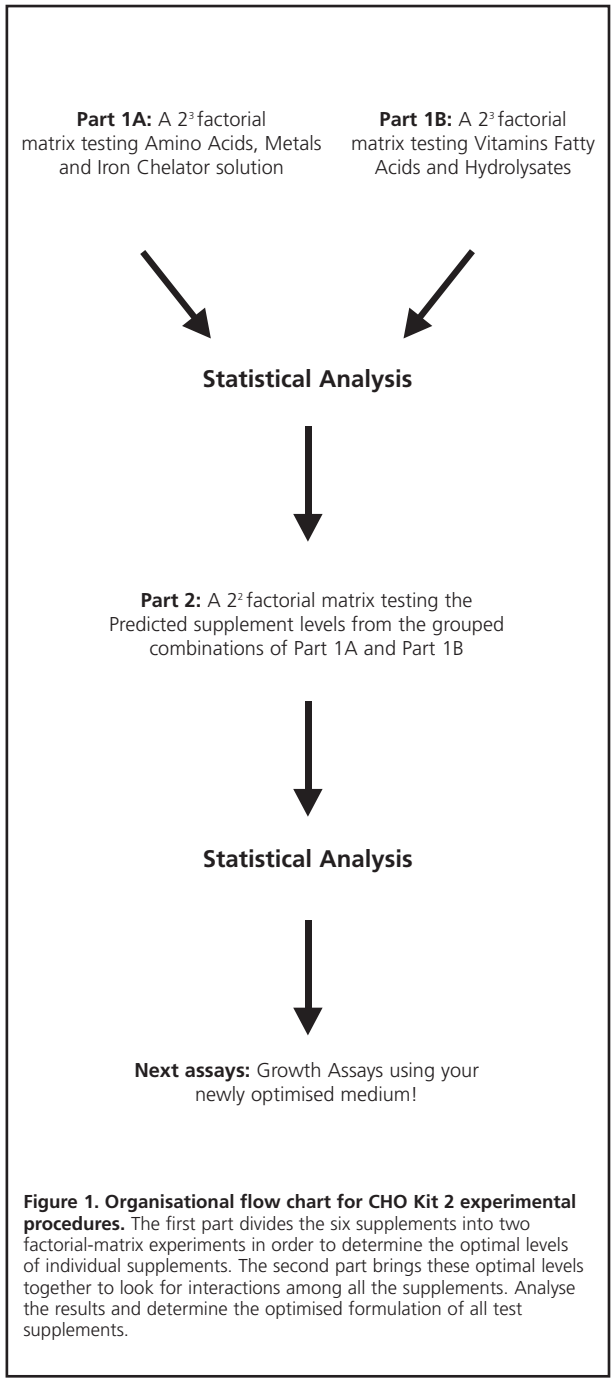

Figure 1. Organisational flow chart for CHO Kit 2 experimental procedures. The first part divides the six supplements into two factorial-matrix experiments in order to determine the optimal levels of individual supplements. The second part brings these optimal levels together to look for interactions among all the supplements. Analyse the results and determine the optimised formulation of all test supplements.

Optimised Media Components Increase Cell Growth and Protein Production

The kit consists of one concentrated, chemically defined basal medium. It is deficient in several nutrients so that any component may be added back for optimisation. Several basal supplements are included to make a complete medium, but not included in optimisation testing. These supplements are glucose, insulin, salts, iron mix, and sodium chloride. Six concentrated grouped media supplements included for optimisation testing are amino acids, vitamins, iron chelator, fatty acids, hydrolysates and metals.

The CHO-K1 cells were adapted to serum-free conditions prior to the experiments. Cells were inoculated at 5 x 10^4 cells/ml. All assays were run in duplicate in 125ml spinner flasks and stored at 37°C, 5% CO_2, with 80rpm stir speed. Samples were counted on a CASY®-1 cell counter (Scharfe Systems, Reutlingen, Germany) and by the trypan blue exclusion method. Data was then input into Design-Expert® for analysis. From the first two factorial matrix assays using CHO-K1 cells, we obtained two predicted optimal growth formulations. Combining data from the two assays, a new medium was prepared: 50% amino acids, 100% metals, 100% iron, 50% vitamins,100% lipids, and 150% hydrolysates. The cell growth performance of this new medium was tested on CHO-K1 cells (Figure 2). The result clearly shows that CHO-K1 cells grew to a 1.5-fold higher cell density in this new medium than in the original Base Medium or in the two competitors' media tested.

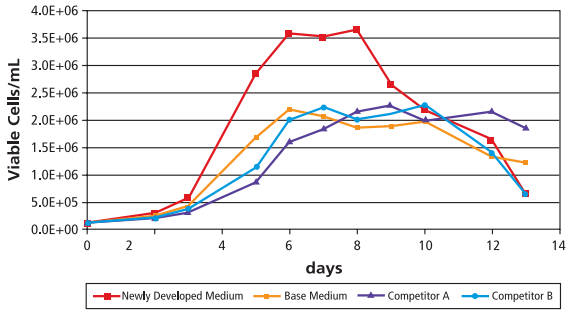

Figure 2. A comparison of the newly developed medium, the original base medium, and two competitors. A new optimised medium was formulated according to the best-predicted values from Design Expert® analysis. Cultures grown in this formulation demonstrated a 1.5-fold increase in cell growth for CHO-K1 cells over the original Base Medium and the two competitors' media.

Kit Provides Medium Optimisation for Several CHO Clones

We have tested this kit using several CHO cell lines to statistically predict the most optimal levels of each component for unique medium supplementation. Our results demonstrate that using this medium optimisation kit and Design Expert® software on diverse CHO cell lines can generate different optimised media formulations (Table 1). Overall, this kit provides a novel approach to medium development, allowing the customer to easily perform factorial-matrix experiments while reducing the time and effort needed to optimise a medium for a particular CHO clone.

		CHO-K1	IgG Clone 1	IgG Clone 2	Alk Phos
Group A	Amino Acids	50%	150%	100%	50%
	Metals	100%	25%	175%	175%
	Iron 1	100%	70%	100%	100%
Group B	Vitamins	50%	50%	150%	100%
	Fatty Acids	100%	150%	150%	100%
	Hydrolysates	150%	150%	150%	150%
Fold Increase in Productivity		n.a.	4.3X	3.7X	2.2X

Some of the components showed similar results between the cell lines. For example, hydrolysates were seen to be beneficial in all clones at 150%. Most of the components showed a difference overall in each cell line, demonstrating that CHO Kit 2 provides diverse nutritional requirements for several cell lines.

Table 1. Comparison of the most optimal medium supplementation values for four CHO clones.

Ordering Information

Prod No.	Description	Pack Size
C4364	CHO Kit 2	1 kit

For further details on these products or to order a copy of our CHO Platform Brochure, please visit our website at sigma-aldrich.com/cellculture and click on 'Cell Culture Literature'

CHO CD-3

CHO CD-3 Chemically- Defined Medium Sustains Better Growth than Hydrolysate-Containing Media in Multiple CHO Recombinant Cell Lines

Scott Ross, Joe Sexton, Nan Lin, and Matthew Caple

Sigma-Aldrich Corporation, St. Louis, MO, USA

- **Chemically-defined and animal component-free**
- **Unsurpassed growth and productivity as compared to hydrolysate-containing formulations**
- **Quick and efficient adaptation of a wide variety of CHO clones directly into chemically-defined formulation**
- **Directly scalable to larger stirred-tank bioreactor systems**

Introduction

Chinese Hamster Ovary (CHO) cells are the most frequently used expression system for the production of recombinant proteins that require post-translational modification to express full biological function. Recombinant CHO clones can be grown in traditional serum-supplemented medium to the most sophisticated chemically-defined animal component-free medium. As the number of recombinant therapeutic proteins produced in CHO systems increases, the methods used to produce them are facing increased regulatory scrutiny. Consequently, chemically-defined formulations are increasingly preferred by biopharmaceutical clients using CHO cells for drug production.

The most challenging aspect of developing chemically-defined formulations is the replacement of specific components with suitable and cost-effective chemically-defined substitutes, while maintaining optimal protein productivity and minimising any potential impact to downstream purification processes. By using statistical experimental design and novel analytical methods, we have developed a new chemically-defined CHO medium that achieves these goals.

Meeting the Demand for Chemically-Defined Formulations

CHO CD-3 Medium (Prod. No. C1490) meets all regulatory concerns for the biopharmaceutical industry by eliminating any animal-derived components in its formula. Additionally, all undefined components such as plant derived hydrolysates that could result in batch-to-batch variability have been eliminated and other components have been redeveloped for use in this product. CHO CD-3 Medium, chemically-defined and animal component-free, is designed to deliver optimal cell growth and recombinant protein expression in suspension culture. The medium does not contain hypoxanthine and thymidine to permit its use in dihydrofolate reductase (DHFR) gene amplification systems.

Media Comparison with Competitors' Chemically-Defined and Hydrolysate-Containing Formulations

Sigma-Aldrich's CHO CD-3 Medium was compared to CHO media from two major competitors (A, B), for growth and productivity in 250ml spinner flasks. Competitor A medium is a serum-free CHO medium containing plant-derived hydrolysates. Competitor B medium is a chemically-defined CHO medium. For these studies several CHO clones were used – one producing a monoclonal antibody and the second producing a recombinant protein from a CHO-K1-derived parental cell. The clones were adapted to a different non-animal component, serum-free CHO Medium (Prod. No. C5467) prior to the start of the experiments. Cells were then inoculated at a density of 5 x 10^4 cells/ml and grown in CHO CD-3 Medium or one of the Competitors' formulations. Figures 1 and 3 illustrate that Sigma's CHO CD-3 Medium consistently supports the highest cell density for CHO cell lines producing recombinant antibody (Cell Lines 1 and 2). Figures 2 and 4 show that CHO CD-3 Medium supports equal or better recombinant protein production as compared to the media of Competitors A and B.

Quick Adaption to CHO CD-3 Medium

The newly developed Sigma-Aldrich CHO CD-3 Medium allows easier adaptation from a protein-free formulation as compared to the two competitors' chemically-defined formulations. Multiple CHO clones have consistently

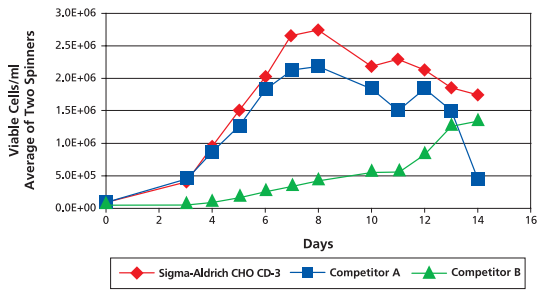

Figure 1. Growth experiment Cell Line 1. This growth curve indicates that Sigma-Aldrich CHO CD-3 Medium attained a maximum viable cell density of 2.75 x 10^6 cells/ml by day 8 as compared to the CHO media of competitors A and B.

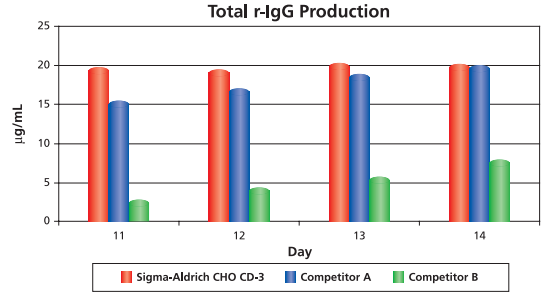

Figure 2. Total r-IgG production Cell Line 1. By day 14 in this clone, the productivity of Sigma-Aldrich CHO CD-3 Medium is equivalent as compared to the hydrolysatecontaining medium of competitor A and the chemically defined medium of competitor B.

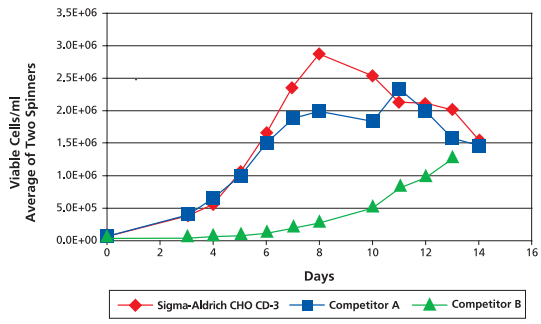

Figure 3. Growth experiment Cell Line 2. The growth curve indicates that Sigma-Aldrich CHO CD-3 Medium attained a maximum viable cell density of 2.86 x 10^6 cells/ml by day 8 as compared to the CHO media of competitors A and B.

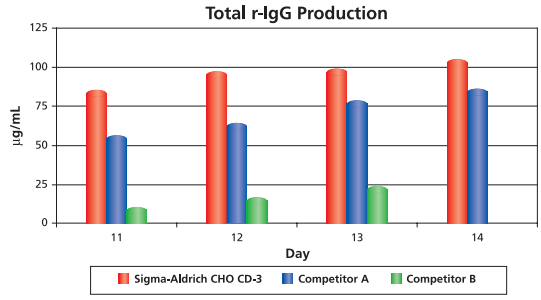

Figure 4. Total r-IgG production Cell Line 2. By day 14 in this clone, the productivity of Sigma-Aldrich CHO CD-3 is the best as compared to the hydrolysate-containing media of competitor A and the chemically defined medium of competitor B.

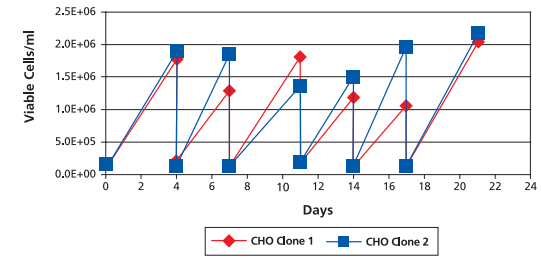

Figure 5. Cell Growth and Adaptation to CHO CD-3 Medium. Different CHO clones grown in serum-free media were directly inoculated into CHO CD-3 Medium. Cells were multiply passaged every 3-5 days. This graph depicts how quickly the cells adapted and are maintained in CHO CD-3 Medium. CHO clones 1 and 2 are fully adapted within the first week.

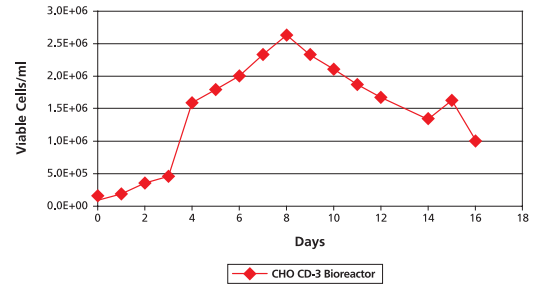

Figure 6. Growth experiment Cell Line 2. The growth indicates that Sigma-Aldrich CHO CD-3 achieved a maximum viable cell density of 2.65 x 10^6 cells/ml by day 8 in a 5L stirred-tank bioreactor.

shown transition from protein-free formulations to the newly developed chemically-defined medium with little to no adaptation delay (Figure 5). Most clones have shown immediate adaptability by direct inoculation into Sigma-Aldrich's CHO CD-3 Medium with growth comparable to protein-free formulations. Several competitive formulations need extensive adaptation time, which in some cases can take up to several months.

Directly Scalable to Stirred-Tank Bioreactors

CHO CD-3 Medium has been designed for the ultimate user goal of scalability to large volume stirred-tank bioreactors. CHO cell cultures can be directly transferred from CHO CD-3 containing spinner flasks to 5L bioreactors. Under sub-optimised non-fed batch bioreactor conditions, several CHO clones have shown similar cell growth and recombinant protein production as previously seen in the spinner flasks (Figures 6 and 7). The new version of CHO CD-3 makes it easy to scale-up to larger bioreactor systems. All work described here was completed in 125-250ml spinner cultures with sequential development in 5L bioreactors for confirmation of scalability. Our studies have shown that direct inoculation into 5L bioreactors deliver equivalent growth and productivity with no additional formulation adjustments. This allows for quick movement from clonal development to pilot scale with no impedance from the medium. This was repeated with several cell lines to identify any minute differences relative to bioreactor cell culture conditions. This will allow for process development to achieve quick scale-up when this formulation is moved from pilot scale into final production scale.

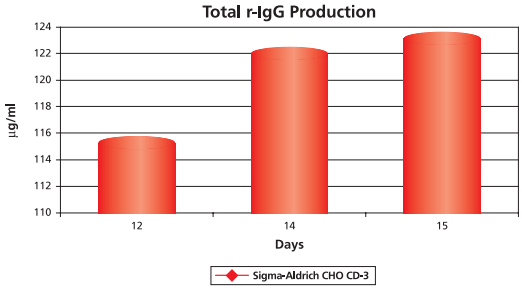

Figure 7. Total r-IgG production Cell Line 2. The productivity of Sigma-Aldrich CHO CD-3 demonstrates the direct scalability into larger bioreactor systems. Total productivity for Cell Line 2 is equivalent to small-scale spinners.

Ordering Information

Prod. No.	Description	Pack Size
C1490	CHO CD-3 Medium, Chemically-Defined, Animal Component-Free	1L 6 x 1L

For more information *on Sigma-Aldrich's range of CHO Platform products, please visit our website at* ***sigma-aldrich.com/cellculture***

NEW Animal Component-Free, Protein-Free Insect Medium

Development of a New Animal Component-Free, Protein-Free Medium for Sf21 and Sf9 Insect Cell Lines

Z.W. Deeds, H. George, F. Swartzwelder, and M.V. Caple

Sigma-Aldrich Biotechnology, Saint Louis, USA

Abstract

The Baculovirus Expression Vector System[1] (BEVS), when used with insect cell lines such as Sf21 or Sf9 (derived from *Spodoptera frugiperda*), is a powerful tool for producing recombinant proteins. Traditionally, insect cells are grown in media that contain serum or other animal-derived products. As more recombinant proteins are being employed as therapeutic agents, the methods implemented in their production are coming under increasing regulatory scrutiny. A major area of concern is the presence of animal-derived components in media used to culture cells for recombinant protein expression[2]. To address this matter, a new animal component-free (AF) insect cell culture medium has been developed. One of the foremost challenges in creating an animal component-free insect medium is the replacement of the traditional lipid source, methyl ester fatty acids from cod liver oil, with a non-animal derived substitute. Experiments utilising factorial matrix design were used to solve this problem. Further medium optimisation and replacement of animal-derived components led to the development of TiterHigh™ Sf Insect Medium (Prod. No. I5408). TiterHigh™ Sf supports high cell densities (>2.0 x 10^7 cells/ml with Sf21 cells in shaker culture), high wild-type and recombinant AcMNPV titers, and high level recombinant protein production using BEVS. Finally, with the utilisation of TiterHigh™ Sf Insect Medium, regulatory concerns associated with the use of animal-derived components have been eliminated.

Introduction

The utilisation of insect cells for the production of recombinant proteins has recently increased due to the relative ease of use and the ability to make large amounts of protein. Advances in creating cell lines with more human-like glycosylation patterns have also led to renewed interest. Cell lines derived from *Spodoptera frugiperda* pupal ovarian tissue, such as Sf9 and Sf21, are routinely used in conjunction with the Baculovirus Expression Vector System (BEVS), which takes advantage of viruses that will infect these cell types. The most commonly used baculovirus is *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV). In BEVS, a non-essential baculoviral gene is replaced with the gene of interest and put under the control of a very late viral promoter, such as polyhedrin or p10. During the very late stages of a recombinant baculoviral infection, large amounts of the desired protein can be produced, sometimes reaching 50% of the total insect cell protein. In response to increased regulatory scrutiny of cell culture media, the need for media that are free of any animal-derived components has come to the forefront. Many animal component-free formulations have been developed for other cell culture platforms, such as CHO and NS0. However, the options for insect cell culture are limited. By using an animal component-free medium, the possible threat of adventitious agent contamination from animal-sourced material can be eliminated.

One of the challenges for the development of an animal component-free insect cell culture medium is the replacement of the lipid source. Traditionally, methyl ester fatty acids that are prepared by the transmethylation of cod liver oil have been used. An identical animal-free replacement would be difficult to design, however the critical lipids could be determined through experimental analysis. Testing lipids one at a time would be time consuming and costly. Instead, a factorial matrix experimental design was applied. This approach allows the researcher to test many variables at once, while reducing of the number of test conditions and uncovering any interactions between the variables. The data from more complex experiments, such as this, cannot be interpreted without the help of a statistical analysis program, such as Design-Expert®.

Additional experimental data with TiterHigh™ Sf Insect Medium is displayed to examine the growth and productivity potential with this new medium.

Materials and Methods

Sigma-Aldrich Corporation (St. Louis, MO, USA) supplied all chemicals, media and solutions unless otherwise stated.

Cell Lines and Baculovirus

Sf9 cells were obtained from the European Collection of Cell Cultures (ECACC), #89070101. Sf21 cells were obtained from Invitrogen, catalogue #11497013.
A recombinant baculovirus, AcP1-57GAL,[3] was used to study recombinant β-galactosidase production β(-gal) with both the Sf9 and Sf21 cell lines.

Culture Media

The media used in this study are TiterHigh™ Sf Insect Medium, Animal Component-Free, Protein-Free and the leading competitors' serum-free, protein-free formulations. A preliminary formulation of TiterHigh™ Sf Insect Medium was used for the animal-free lipids study.

Cell Growth and Recombinant Protein Production Assays

Sf9 and Sf21 cells were routinely cultured in suspension, in TiterHigh™ Sf or the respective competitor's medium,

and were used to seed experiments conducted in 125ml (50ml working volume) disposable Erlenmeyer shake flasks (Corning). Initial cell density was $3x10^5$ viable cells/ml for the growth assays. The productivity assays were infected with a Multiplicity of Infection (MOI) of 5 at a cell density of $1x10^6$ viable cells/ml. All conditions were tested in duplicate. The cells were cultured in a ThermoForma incubator at 27°C and 130rpm shaker speed. Spent medium samples were collected every day for the analysis of nutrients/metabolites. At the same time, total cell number was determined using a Schärfe System Casy 1® Model TTC and viability was assessed using the Trypan Blue Exclusion Method.

Quantification of Recombinant β-galactosidase

β-gal was quantified using Sigma's β-galactosidase Reporter Gene Activity Detection Kit (Prod. No. GAL-A). One-milliliter samples were collected every day and the cells were washed with HBSS (Prod. No. H6648) after centrifugation. The cell lysates were diluted 1:2000.

Discussion

To generate an animal component-free insect cell culture medium, multiple modifications must be made to current formulations. Primarily, the methyl ester fatty acids from cod liver oil need to be exchanged with a defined lipid mixture. Since cod liver oil is an undefined material, experiments had to be conducted to establish which fatty acids were critical for the Sf21 and Sf9 cell lines. In this study, a factorial matrix experiment was used to test multiple fatty acids at once. A full matrix experiment testing all of the chosen lipids in all of the various combinations would be 2^7 (128) conditions, but to simplify the experiment a 2^{7-2} (32 conditions) matrix was performed. This allows the researcher to run only 1/4 of the possible conditions and at the same time keeps the loss of significant data to a minimum. The results (cell growth represented as cell-days) of this experiment were analysed using Design-Expert® and are shown in Figure 1

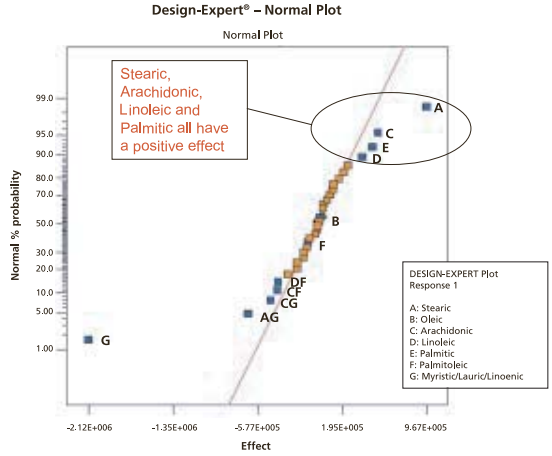

Figure 1. Normal Plot of the relative effects of the lipids tested. This graph is a representation of the relative effects of the lipids tested in a factorial matrix designed experiment (27-2 or 1/4 matrix). The cell growth data from the experiment was analyzed in Design Expert to elucidate the effects of individual or combinations of fatty acids. The points that fall to the right of the line denote a positive response.

as a normal probability plot. In this type of plot the "normal" or insignificant data points lie on a relatively straight line. Any points that do not fall on this line are deemed significant. The points can represent either a specific lipid or a combination of lipids that when used in conjunction have some type of interaction. Those that fall to the left of the line have negative effects, while those that fall to the right have positive effects. The normal plot indicates that stearic, arachidonic, linoleic, and palmitic acids all yielded positive effects on cell growth (Figure 1).

In a subsequent experiment, as depicted in Figure 2, a lipid mixture based on the results from Figure 1 was tested against the cod liver oil fatty acids in a preliminary formulation of TiterHigh™ Sf Insect Medium (Prod. No. I5408). All conditions had very similar growth, indicating that the new animal-free lipid mixture is sufficient to sustain Sf21 and Sf9 cells. At this point the preliminary formulation of TiterHigh™ Sf was animal component-free and also supported levels of cell growth that were close to the level of the competition. Further optimisation led to much improved cell growth (Figure 2).

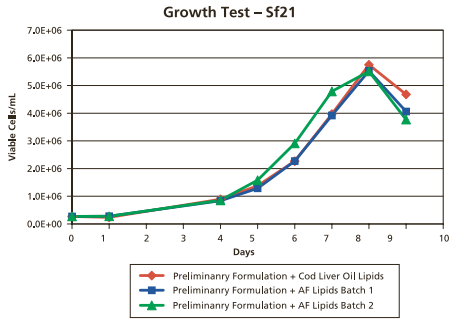

Figure 2. Comparison between the different lipid sources with Sf21 cells. The methyl ester fatty acids from cod liver oil were tested against two batches of the animal-free lipids in shaker cultures using a preliminary formulation of TiterHigh™ Sf Insect Medium. The conditions all preformed similarly.

Figure 3 demonstrates the excellent growth that is attainable in TiterHigh™ Sf Insect Medium with the Sf21 cell line in shaker culture.

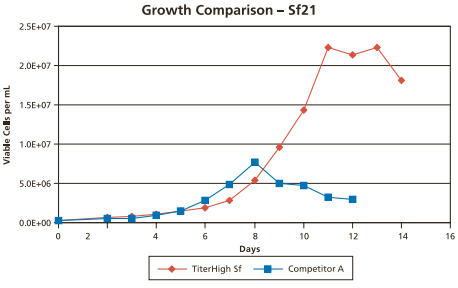

Figure 3. Comparison between TiterHigh™ Sf Insect Medium and the leading competitor for growth with the Sf21 cell line. In this experiment Sf21 cells, grown in their respective media, were seeded in Corning shake flasks (125ml) at 3 x 10^5 viable cells/ml. The results show that TiterHigh™ Sf supports excellent cell growth.

Figures 4 and 5 show that this new medium will also support high level recombinant protein production after baculoviral infection. Additionally, TiterHigh™ Sf will support baculovirus titers of greater than 10^8 PFU/ml (data not shown).

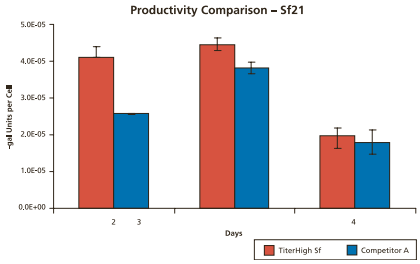

Figure 4. Comparison between TiterHigh™ Sf Insect Medium and the leading competitor for productivity with the Sf21 cell line. In this experiment Sf21 cells, grown in their respective media, were infected with a recombinant (β-gal) baculovirus at an MOI of 5 in Corning shake flasks (125ml) at 1 x 10^6 viable cells/ml. The results show that TiterHigh™ Sf supports high level protein production.

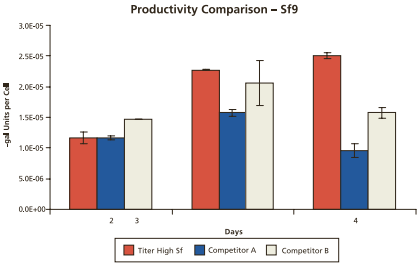

Figure 5. Comparison between TiterHigh™ Sf Insect Medium and the leading competitor for productivity with the Sf21 cell line. In this experiment Sf21 cells, grown in their respective media, were infected with a recombinant (β-gal) baculovirus at an MOI of 5 in Corning shake flasks (125ml) at 1 x 10^6 viable cells/ml. The results show that TiterHigh™ Sf supports high level protein production.

Conclusions

- Using factorial matrix experimental design, an animal-free substitute for the traditional insect medium fatty acid source was successfully developed.
- With the utilisation of TiterHigh™ Sf Insect Medium (I5408), regulatory concerns associated with the use of animal-derived components have been eliminated.
- TiterHigh™ Sf Insect Medium is one of the best commercially available media for growth with the Sf21 and Sf9 cell lines.
- TiterHigh™ Sf Insect Medium also supports excellent recombinant protein production using the Baculovirus Expression Vector System (BEVS).

Acknowledgements

We would like to thank all who contributed to the research and development of TiterHigh™ Sf Insect Medium at the Sigma-Aldrich Life Science and High Technology Center.

References

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Ordering Information

Prod No.	Description	Pack Size
I5408	TitreHigh Sf Insect Medium	1L 6x1L

Cell Line Specification

Sf9
Catalogue no. 89070101
The Sf9 insect cell line is derived from pupal ovarian tissue of *Spodoptera frugiperda.* The cells are highly susceptible to Baculovirus infection and are used in the production of protein products genetically manipulated into Baculovirus vector systems.

Sf9 TitreHigh AC-free
Catalogue no. 05011001
This cell line is a derivative of the Sf9 cell line which has been adapted to TitreHigh Sf Insect Medium (Sigma I5408). TitreHigh Sf Insect Medium is an animal component-free, protein-free formulation designed specifically for the Sf9 and Sf21 *Spodoptera frugiperda* insect cell lines.

Sf21
Catalogue no. 05022801
The Sf21 insect cell line is derived from pupal ovarian tissue of *Spodoptera frugiperda.* The cells are highly susceptible to Baculovirus infection and are used in the production of protein products genetically manipulated into Baculovirus vector systems.

Sf21 TitreHigh AC-free
Catalogue no. 05030202
This cell line is a derivative of the Sf21 cell line which has been adapted to TitreHigh Sf Insect Medium (Sigma I5408). TitreHigh Sf Insect Medium is an animal component-free, protein-free formulation designed specifically for the Sf9 and Sf21 *Spodoptera frugiperda* insect cell lines.

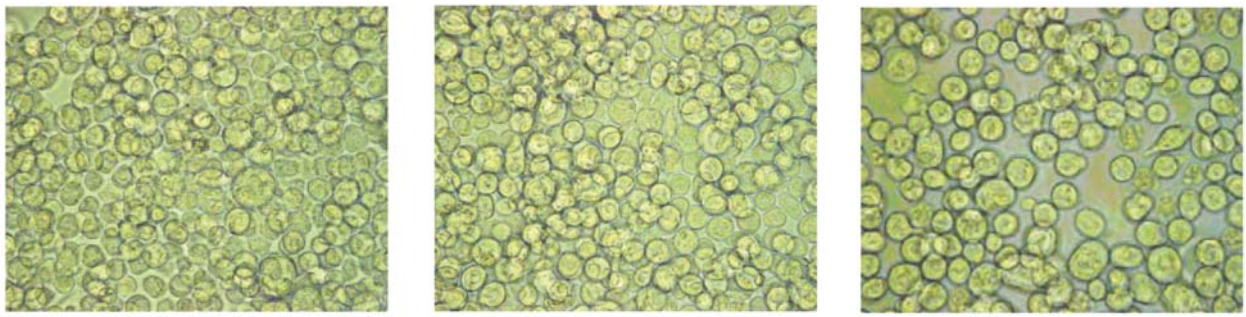
A Complete Line of Insect Cell Expression Products from Sigma-Aldrich

DiamondBac™ Baculovirus DNA, Baculovirus Transfer Vectors and Escort™ Transfection Reagent

DiamondBac™: The Baculovirus System that Shines

DiamondBac™ Baculovirus DNA is a linearised modified baculoviral DNA derived from AcNMPV (*Autographa californica* nuclear multiple polyhedrosis virus) that allows rapid and convenient generation of recombinant baculovirus clones. DiamondBac™ has been engineered to ensure complete linearisation of the parental viral genome and contains a lethal deletion of the essential ORF1629 sequences. Therefore, parental viral DNA background is eliminated and recombination efficiencies approach 100% upon co-transfection with an appropriate transfer vector. DiamondBac™ is compatible with most commercially available polyhedrin-based transfer vectors such as the pPolh baculovirus transfer vector line.

- **Reduce Time and Effort in Plaque Assays** – Nearly 100% recombination efficiency
- **High Level Expression** – Up to a 6-fold increase in overall protein production
- **Enhanced Protein Folding** – Codes for a protein chaperone that aids in folding and proper disulfide bond formation
- **Increased Cell Viability** – Deletion of the p10 gene in the viral DNA coupled with use of recommended ESCORT™ or ESCORT™ IV Transfection Reagents leads to optimal cell viability
- **Economical** – Achieve a greater number of transfections per µg of viral DNA



Untreated Control | **DiamondBac™** | **Competitor**

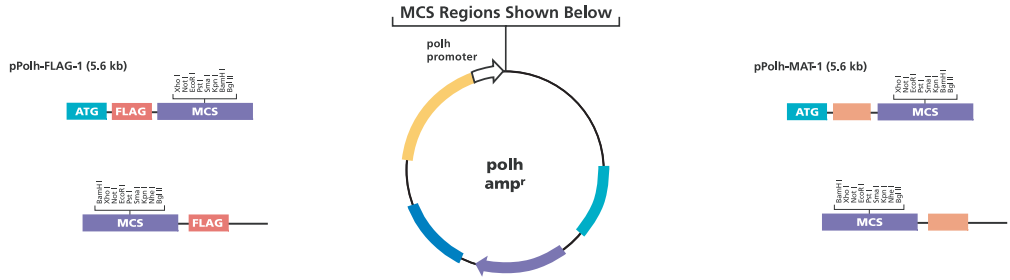
Figure 1. No Detectable Parental Background with DiamondBac Baculovirus DNA Sf21 cells are shown are at day 5 after infection with P1 linear viral stocks. The DiamondBac sample shows no sign of infection compared to the untreated cells while the competitor sample shows classic signs of infection.

Baculovirus Transfer Vectors

Baculovirus Transfer Vectors

pPolh transfer vectors are for the construction of recombinant baculovirus used in insect cell expression systems. The strong polyhedrin(polh) promoter results in high-level expression of target genes during the very late phase of infection. The AcNPV ORF 603 and ORF1629 regions flank the polh promoter and MCS for homologous recombination with baculoviral DNA and generation of recombinant baculovirus. pPolh vectors also contain the pUC origin of replication and β-lactamase gene ampr for propagation and selection in bacterial cells. The following baculovirus transfer vectors are offered as N- or C-terminal FLAG® or MAT™ (Metal Affinity Tag) vectors for convenient downstream detection and purification of recombinant protein.

- Expression of recombinant fusion proteins at high levels in insect cells
- High-level detection and purification using ANTI-FLAG® antibodies, resins, and 96-well plates
- New 7 amino acid MAT tag provides for powerful one-step purification using HIS-Select™ Nickel and Cobalt affinity gels
- Compatible with commercially available ORF 1629 deleted baculoviral DNA



ESCORT™ Reagents: For High Efficiency Transfection of Insect Cells

ESCORT Transfection Reagent

ESCORT has been demonstrated to be an ideal transfection reagent for the transfection of Sf9 insect cells. Transfection of Sf9 cells using ESCORT results in higher efficiencies, better cell viability and requires less reagent and DNA compared to other transfection reagents. In addition, ESCORT may be used to transfect a wide variety of cell lines. For general transfection of cell lines, 5µl is required for a 35mm culture dish while 15µl is sufficient for a 60mm dish.

Features & Benefits

- High efficiency transfection of Sf9 cells
- Low toxicity
- Economical
- Efficiently transfects a wide variety of cell lines

ESCORT IV Transfection Reagent

Optimal transfection of both Sf9 and *T.ni.* insect cells is achieved using ESCORT IV. Using ESCORT IV, higher levels of transfection and resulting expression were seen in these cell lines compared to competitor products while maintaining lower levels of toxicity. For general transfection of cell lines, peak activities were achieved using 4-6µg of ESCORT IV per µg of DNA in 35mm dishes.

Features & Benefits

- Reagent of choice for Sf9 and *T.ni.* insect cells
- Low toxicity
- Recommended for cultured cell lines
- Useful for a wide variety of cell types

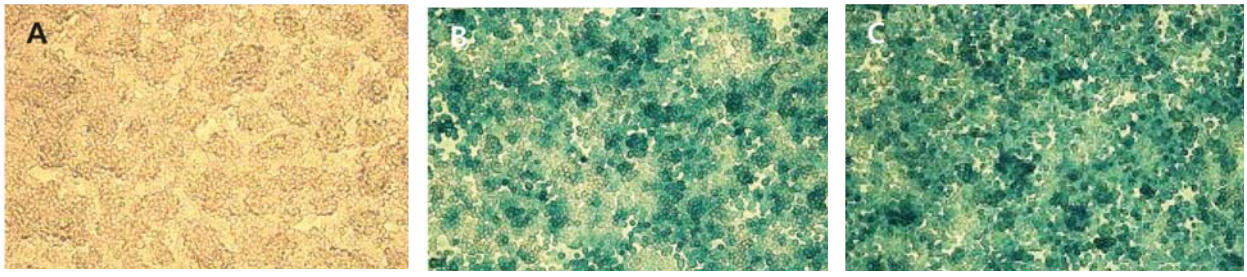


Figure 2. Transfection of Sf9 insect cells with ESCORT and ESCORT IV Sf9 cells in exponential growth phase were seeded at a density of 3 x 10^6 in 60mm dishes. After allowing the cells to attach for 1 hour, the serum-containing medium was removed. The cells were transfected in serum-free medium with 3µg of plasmid DNA containing a constitutively expressed LacZ gene. 12µl of lipid reagent was used to transfect 3µg of plasmid DNA. After addition of the lipid: DNA mixture, the cells were incubated for 5 hours. Following incubation, the medium was replaced with serum containing medium. After a 40 hour incubation period, the cells were stained for the presence of β-galactosidase. Panel A shows staining of untransfected Sf9 cells. Panel B contains Sf9 cells transfected using ESCORT while panel C demonstrates high efficiency transfection of Sf9 cells using ESCORT IV.

Ordering Information

Prod. No.	Description	Pack Size
D6192	DiamondBac™ Baculovirus DNA	5µg
T6824	pPolh-FLAG™-1 Transfer Vector	20µg
E6155	pPolh-FLAG™-2 Transfer Vector	20µg
T6699	pPolh-MAT™-1 Transfer Vector	20µg
T6574	pPolh-MAT™-2 Transfer Vector	20µg
E9770	ESCORT™ Transfection Reagent	0.25ml 0.5ml
L 3287	ESCORT™ IV Transfection Reagent	1ml
G1397	Gentamicin Solution	10ml 100ml
I5408	TitreHigh™ Sf Insect Medium	1L 6 x 1L

For additional information *on vectors and transfection reagents from Sigma-Aldrich, please visit our website at* ***sigma-aldrich.com/cloning***

Whole Genome Amplification from Archived Formalin-fixed, Paraffin-embedded Tissues

Rosemarie Walter, James Eliason, Yezhou Sun, Joseph Cortese, and Richard Everson

Asterand, Inc., Detroit, MI, USA. Barbara Ann Karmanos Cancer Institute. Wayne State University School of Medicine, Detroit, MI, USA.

- **High yield from limited template – Amplify nanogram amounts of genomic DNA into microgram yields in less than three hours**
- **Preserve precious samples – Only ten nanograms of starting material required**
- **Amplify DNA from any source – Suitable with numerous sources of DNA including FFPE, buccal swabs, cell culture, plants and bacteria**
- **Unlimited genetic analysis – Compatible with many downstream applications such as TaqMan® and BeadArray® assays**

Introduction

Archived formalin-fixed and paraffin-embedded (FFPE) tissue collections represent invaluable resources for studying pathogenesis of cancer and a variety of other diseases. The possibility of retrospective analysis of pathogenic as well as normal specimen and correlation of the molecular finding to disease and drug development is a critical issue. The accessibility of nucleotides from FFPE tissue is limited, because of extensive cross-linking of all tissue components. The DNA fragmentation depends on the tissue type and the fixation condition.

With the development of whole genome amplification (WGA) techniques, large quantities of DNA can be generated from limited starting quantities. Procedures have been used such as primer extension pre-amplification (PEP) with random 15-mer primers to amplify genomic DNA from single cells or low available amounts of DNA.[1] Other strategies like linker adaptor PCR,[2] tagged PCR,[3] and degenerate oligonucleotide primed PCR (DOP)[4] were designed, improving the yield, but the coverage of the genome in the amplification products was not optimal. Applying multiple displacement amplification (MDA)[5] with random primers and ϕ29 DNA polymerase ensured accurate whole genome amplification from small amounts of clinical samples, but typically has not been successful with FFPE tissue. We evaluated the ability of the GenomePlex™ WGA Kit developed by Rubicon Genomics, Inc. (Ann Arbor, MI, USA) to amplify fixed tissue. GenomePlex WGA is based on random fragmentation of genomic DNA and amplification by PCR using ligated adapters to create the OmniPlex™ Library.[6] The resulting library can be amplified more than thousand-fold.

DNA Extraction and Whole Genome Amplification

One or two sections from FFPE prostate samples (10-15 years old) were cut 5µm thick and genomic DNA was extracted following the protocol shown in Figure 1.

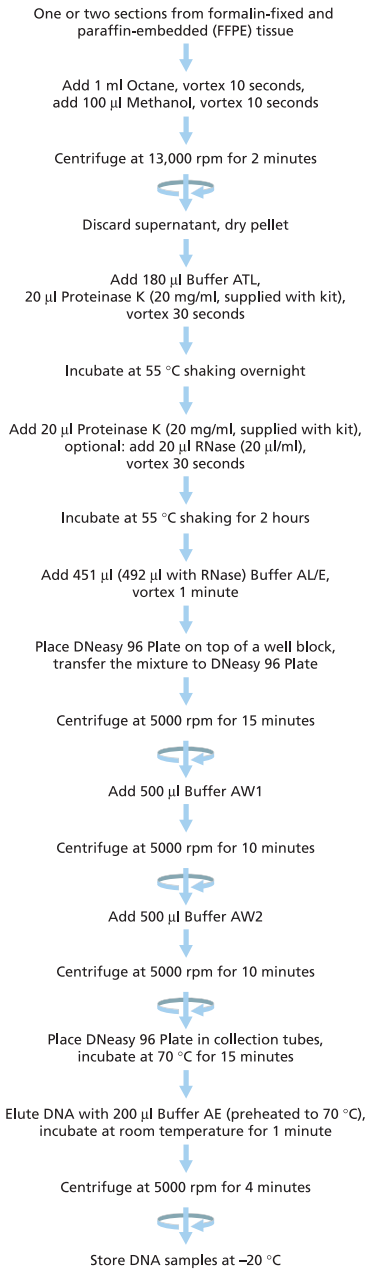


Figure 1. Amplification plots for different real-time PCR assays. After each PCR cycle fluorescent intensities were normalized to the maximum intensity. *SaFigure 1. DNA extraction from formalin-fixed and paraffin-embedded tissue. Modified procedure using commercially available kit for high-throughput DNA modification samples are duplicates.

Quantification of the DNA was determined using the PicoGreen™ assay (Molecular Probes, Eugene, OR, USA). Samples were normalised to 20ng DNA in 10µl TE (10mm Tris, 1mm EDTA at pH8.0). For complete and accurate whole genome amplification, the GenomePlex Kit was applied and an OmniPlex library created according to the supplied protocol. Quantitative real-time PCR amplifications were performed on an iCycler™ thermocycler (Bio-Rad, Hercules, CA, USA) with SYBR® Green dye (Cambrex BioScience Rockland, Rockland, ME, USA) detection. To each sample well 0.75µl of SYBR Green (1:1000 dilution in TE) and 0.75µl of Fluorescein Calibration Dye (1:1000 dilution in TE) (Bio-Rad, Hercules, CA, USA) were added and amplified using a two step program: initial denaturation at 95°C for three minutes, followed by 16 cycles with denaturation at 94°C for 15 seconds and primer annealing/extension at 65°C for two minutes. Amplification products were purified using a commercially available kit and quantified by spectrophotometer. The average yield was 4µg with an average amplification of 200-fold amplification (data not shown).

The real-time PCR amplification cycle plot was displayed with the background subtracted and variable baselines. In order to interpret the curves and make them more comparable, the individual readings for each sample well were displayed, copied and transferred to a spreadsheet program. From each data point on a particular sample curve the lowest relative fluorescent units (RFU) value was subtracted and the fluorescence intensity was normalised to the maximum of the sample curve (Figure 2). Samples qualified for downstream applications when their threshold values (C_T) were within four cycles of the control DNA, unless the amplification continued not too far into saturation. The amplified tissue samples were normalized to 4ng/µl for testing.

Statistical Approach

A multidimensional approach, based on Design-of-Experiment (DOE) principles, to optimising medium components. We use several methods to test multiple variables simultaneously, reducing the number of test conditions without a significant loss of information to speed the development process.

Creating Better Results Through a Better Process:

- Reduced experimental iteration resulting in better labor efficiency
- Early definition and recognition of interactions between components
- Better optimisation of interacting components
- More objectively defined endpoints
- Reduced development cycles of 3 to 9 months

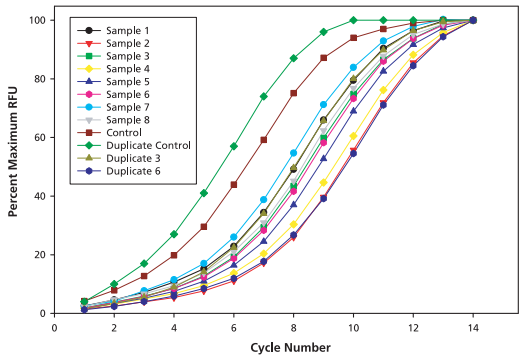


Figure 2. Amplification plots for different real-time PCR assays. After each PCR cycle fluorescent intensities were normalised to the maximum intensity. *Samples are duplicates.

Summary

The GenomePlex WGA Kit developed by Rubicon Genomics and offered exclusively by Sigma-Aldrich is ideal for isolation of human DNA from tissue samples like blood or mouthwash, fresh frozen and formalin-fixed and paraffinembedded. It is a robust method for generating limited DNA into microgram quantity within less than three hours. We are currently evaluating the success of the amplified products for genotyping and other molecular assays.

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Ordering Information

Prod No.	Description	Pack Size
WGA-1	GenomePlex WGA Kit, 50rxn	1 kit

Ask ECACC ....

Ask ECACC .... Focus on Epstein Barr Virus (EBV) lymphocyte transformation

ECACC has provided an EBV lymphocyte immortalisation and cell banking service to human genetic research in the UK since 1986. During this period ECACC has provided support to hundreds of research groups and amassed cell banks representing up to 100,000 donor subjects from over 800 different genetic orders. ECACC receives a diverse range of technical enquires and in this issue we have focused on EBV lymphocyte transformation.

1 How does EBV transformation work?
The virus binds to the CD21 receptor on the B cell and enters the cell. The virus DNA is then transferred to the nucleus where, by the action of various oncogenes the cell develops the capacity to reproduce indefinitely, thus a lymphoblastoid cell line (LCL) is generated.

2 Will EBV affect subsequent genetic analysis of genomic DNA from LCLs?
The effect of EBV transformation on genomic DNA is uncertain, although it is believed that the viral DNA remains episomal. DNA from LCLs is suitable for studies on both transcriptional and translational products. Unlike whole genome amplified DNA, DNA from LCLs can be used in the study of DNA methylation, very large DNA fragments, repetitive gene sequences and telomeric repeats.

3 Are all blood samples transformable?
This depends on the quality of the sample, the age of the individual, their disease status and treatment regimes. A small proportion of the population (1-2%) may also be inherently "un-transformable".

4 What transformation success rates can I expect?
The average success rate for EBV transformation at ECACC is currently >95% at first attempt. This will vary between specific projects and the quality of incoming blood specimens.

5 How can I ensure a good quality blood sample?
Blood should be sent to ECACC as soon as possible after collection, preferably within 48 hours. We recommend the use of Acid Citrate Dextrose (ACD) as an anticoagulant, and a minimum sample volume of 6ml. The blood should be kept at ambient temperature (20˚C) after collection and during despatch. The blood tubes need to be packed correctly and safely and never refrigerated. Detailed information on how to send samples can be found at **www.ecacc.org.uk** under "Services".

6 Does the length of storage of frozen peripheral blood lymphocytes (PBLs) affect transformation success rate?
At ECACC, PBLs stored safely at a constant temperature in Liquid Nitrogen storage vessels for greater than 10 years have transformed with no apparent reduction in success rate.

7 Can I store PBLs that I have separated myself at –80°C? If so, for how long?
We do not recommend storing PBLs at –80°C. Long term storage of cell lines and PBLs can only be guaranteed by storage at liquid nitrogen temperatures. However, if there is no alternative, then –80°C storage should be employed only for weeks rather than months and never for years.

8 Do LCLs remain diploid (or genome remain stable) on long term culture?
LCLs may be polyploid rather than diploid (i.e. each cell will have more than two copies of each chromosome). Once polyploidy is established, cells will remain relatively genetically stable.

9 What safety level should LCLs be handled at?
Unless there is information to the contrary (e.g. the patient is known to be Hepatitis B or HIV positive) LCLs should be handled at UK ACDP containment level 2 (CL2) or equivalent, to contain any residual risk from potential adventitious agents that the cell line may be harbouring.

10 Can LCLs propagate HIV or Hepatitis B or C?
HIV primarily infects T cells and as far as we can determine from the literature, there is no cell line currently available that will propagate Hepatitis B and C viruses. ECACC is able to process blood samples and prepare LCLs from patients with HepB, HepC or HIV at ACDP containment level 3 by prior arrangement.

11 How can I be sure that the cell line DNA is identical to the DNA I have extracted direct from the patients blood?
Upon arrival at ECACC, before the blood sample is separated, a small quantity of blood from each patient is stored on a blood spot card. This allows for verification of the LCL against patient DNA using Single Tandem Repeat (STR) Multiplex PCR Profiling. As part of routine Quality Assurance, ECACC tests 5% of LCLs against source DNA. However, EBV transformation is a protracted, highly manual process, and as such, a small degree of error (usually < 1%) is possible.

If you have any technical questions relating to this service please feel free to call ECACC on **+ 44 1980 612684** or email **ecacc.technical@hpa.org.uk.** Alternatively, visit the ECACC website **www.ecacc.org.uk.**

To order direct from ECACC

Contact our customer service team

For direct sales, sales enquiries, delivery and pricing details contact ECACC between normal business hours, Monday – Friday, 8.45 – 17.00 (UK Time).

Orders can be placed by:

Fax: + 44 (0)1980 611315
Website: www.ecacc.org.uk
Email: ecacc@hpa.org.uk
Mail: European Collection of Cell Cultures, Health Protection Agency, Centre for Emergency Preparedness and Response
Porton Down, Salisbury, Wiltshire, SP4 0JG, U.K.

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