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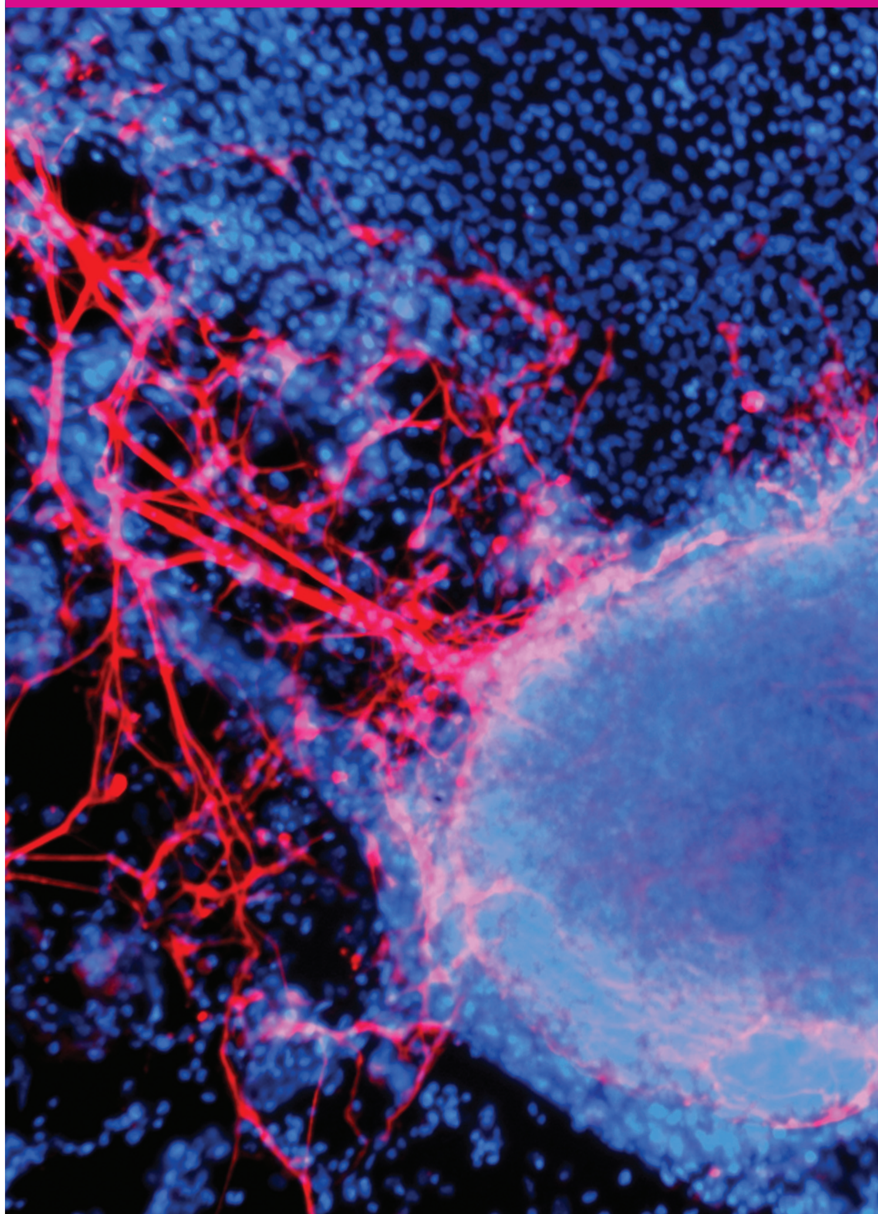
THE NEWSLETTER FOR CELL BIOLOGY RESEARCHERS



INSIDE:

2 Improved Method for Adenovirus and Lentivirus Purification using the Fast-Trap™ Virus Purification and Concentration Kit

5 Progenitor Cell Targeted Culture Media Improve Primary Culture of Epithelial Cells

8 ENStem™-A Adherent Human Neural Progenitors: A New Source of Primary Human Neural Cells

11 Embryoid Body Differentiation Methods for Neurogenesis and Adipogenesis

13 New in Apoptosis Imaging: Dual Detection of Self-Execution and Waste-Management

16 New Products

Cover Photo: Embryonic stem cells differentiated as intact embryoid bodies (EBs) give rise to complex networks of differentiated neurons with high levels of branching. Red: neurons labeled with βIII-tubulin antibody. Blue: cell nuclei labeled with DAPI.

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Improved Method for Adenovirus and Lentivirus Purification using the Fast-Trap Virus Purification and Concentration Kit

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Abstract

Highly purified virus is necessary for many viral vector applications such as vaccine production and genetic modification. Crude virus preparations often contain cellular debris and proteins from culture media that are toxic to target cells and can cause immunogenic reactions *in vivo*. Conventional virus purification methods based on sucrose or cesium gradient ultracentrifugation are time-consuming, difficult, and require access to special instrumentation. These methods also often result in low virus recovery.

Here we report a fast and easy method for adenovirus and lentivirus purification using the Millipore Fast-Trap Virus Purification and Concentration Kit. Both adenovirus (Cat. No. FTAV 000 03) and lentivirus (Cat. No. FTLV 000 03) kits contain the necessary reagents and filter devices to accommodate the entire virus purification workflow (Figure 1). The purification is membrane-based and utilizes a closed vacuum-driven device. High recoveries of purified viable viral particles are typically achieved in under two hours.

Introduction

Viruses are an increasingly valuable tool in bioresearch. A variety of virus-derived vectors have been developed for improved gene transfer in mammalian cells. Recombinant viral vectors have widespread use in the field of gene therapy[1] and for vaccination purposes[2]. For both adenovirus and lentivirus, purifying the virus after it is propagated in the host cell is a critical step. Adenoviral and lentiviral vectors have traditionally been purified by density gradient centrifugation[3,4]. However, density gradient techniques are lengthy, tedious, and often result in low virus recovery. In addition, this purification method requires the use of

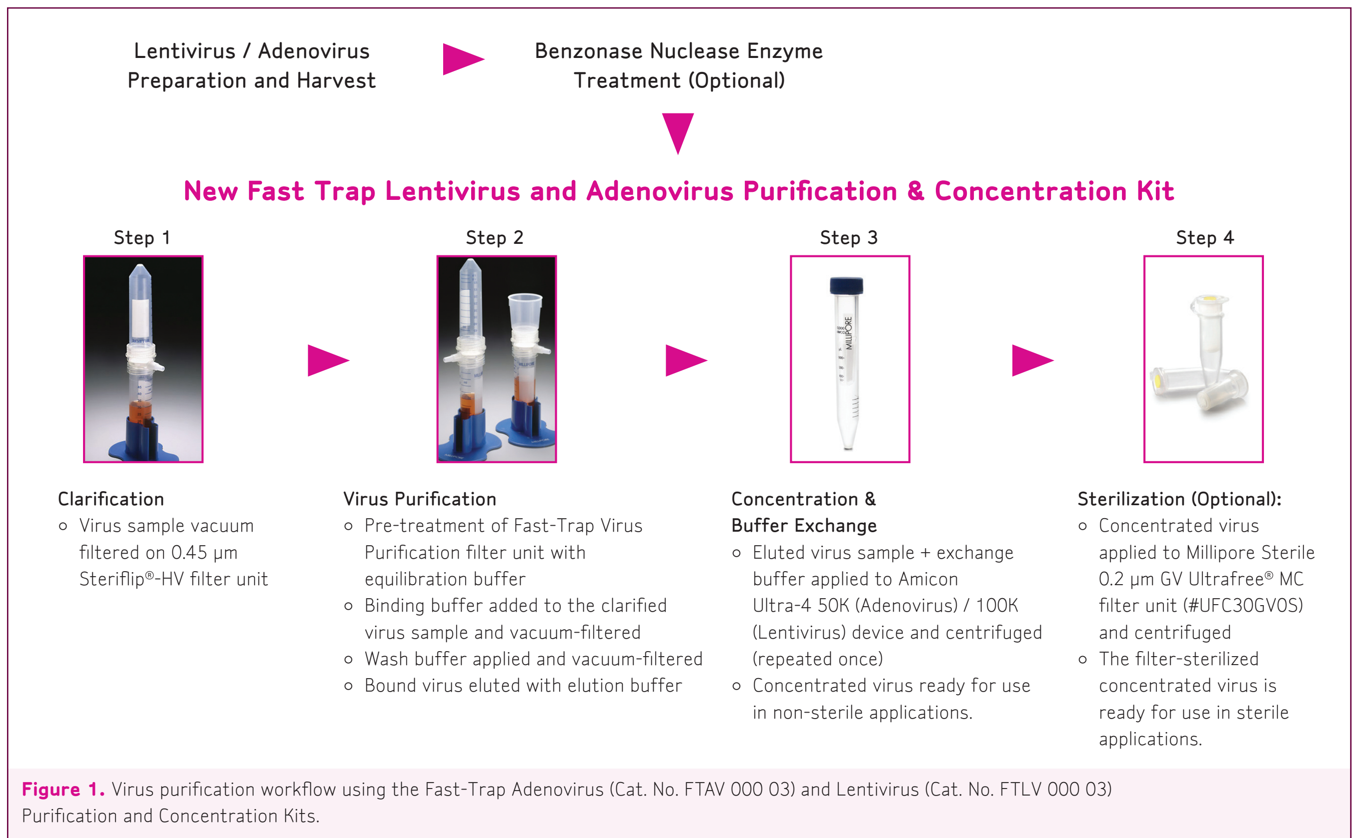

Figure 1. Virus purification workflow using the Fast-Trap Adenovirus (Cat. No. FTAV 000 03) and Lentivirus (Cat. No. FTLV 000 03) Purification and Concentration Kits.

ultracentrifuges which are expensive and not common equipment in an average laboratory.

Recently column or membrane chromatography methods[5,6] were developed for virus purification. The amount of time required to complete these methods is much less than by traditional methods, typically under two hours. Most lab-scale chromatographic virus purification devices are syringe filter- or column-based. However, syringe filter-based methods require assembly and disassembly which can lead to messy and potentially hazardous handling conditions.

Here we report a significantly improved solution for virus purification. A new membrane was developed to selectively bind and elute adenoviruses and lentiviruses using specially formulated buffers. To add safety and ease of handling, this membrane is housed in a newly engineered, vacuum-based device: the Fast-Trap Virus Purification filter unit. Both Adenovirus and Lentivirus Fast-Trap Purification kits perform as good as or better than similar products from other manufacturers in terms of processing time, recovery, purity, and capacity. The entire protocol, including clarification of crude virus, purification of the virus, buffer exchange, and concentration, can be accomplished in under two hours and results in a concentrated high titer, pure virus in the buffer of choice.

Results & Discussion

ADENOVIRUS PURIFICATION

Ad5.CMV5-GFP (Q-BIOgene, Cat. No. ADV0032), a serotype 5 adenovirus expressing GFP under the control of a modified CMV promoter, was purified using the components of Millipore's Fast-Trap Adenovirus Purification and Concentration Kit.

Ad5.CMV5-GFP viral stock was amplified in HEK293 cells. Two to four days after infection, when cells detach and round up, the cells were harvested and pelleted. Cell pellets were resuspended in fresh media and subjected to three freeze/thaw cycles to liberate the adenovirus from the HEK293 cells. The crude adenovirus was then clarified using a 0.45 µm Steriflip-HV device and mixed with 10X binding buffer. The viral mix was loaded onto the Fast-Trap Adenovirus Purification device and viral particles were bound to the membrane using vacuum filtration. After washing, the bound virus was eluted using an elution buffer. For concentration and buffer exchange, the eluted purified adenovirus was added to a 50 kDa NMWL Amicon® Ultra-4 filter unit along with exchange buffer of choice. The device was centrifuged and an additional volume of exchange buffer was added. After a final centrifugation step, the purified, concentrated adenovirus was collected and assessed for titer and purity.

Starting material (clarified adenovirus), flow-through (unbound material) and the final purified adenovirus were titered using the $TCID_{50}$ assay to determine the amount of viable virus particles that was bound and recovered. On average 90 - 100% of the adenoviral particles were bound to the membrane and more than 60% of the adenovirus was recovered in the elution. The whole purification workflow described above could be accomplished in about 30 minutes when low titer adenoviral samples were processed. Samples with higher titers (maximum capacity is approximately 1 x 10^{13} total adenoviral particles) could be processed in under two hours. Purity of eluted adenovirus samples was assessed using SDS-PAGE stained with Coomassie Blue (Figure 2). The majority of contaminating cellular and media proteins were removed (Figure 2, lane 1 and 2) and were not present in the elution fraction (Figure 2, lane 3).

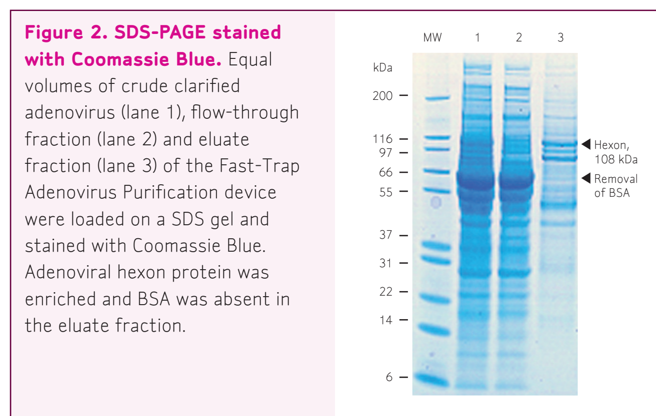

Figure 2. SDS-PAGE stained with Coomassie Blue. Equal volumes of crude clarified adenovirus (lane 1), flow-through fraction (lane 2) and eluate fraction (lane 3) of the Fast-Trap Adenovirus Purification device were loaded on a SDS gel and stained with Coomassie Blue. Adenoviral hexon protein was enriched and BSA was absent in the eluate fraction.

The performance of the Fast-Trap Adenovirus Purification and Concentration Kit was compared to the traditional method of adenoviral purification, double cesium chloride (CsCl) gradient ultracentrifugation. Equal amounts of crude adenovirus were provided for each purification. Double CsCl gradient purification, processed by a specialized viral vector core facility, recovered 45% of the virus while Millipore's Fast-Trap purification provided 70% virus recovery (Table 1).

	Fast-Trap Adenovirus Purification and Concentration kit	Double CsCl Gradient Ultracentrifugation
Amount crude adenovirus (ivp)	5.81 x 10^9	5.81 x 10^9
Amount purified adenovirus (ivp)	4.05 x 10^9	2.64 x 10^9
% virus recovery	70	45

Table 1. Adenovirus purification results using Fast-Trap Adenovirus Purification and Concentration Kit and double CsCl gradient ultracentrifugation.

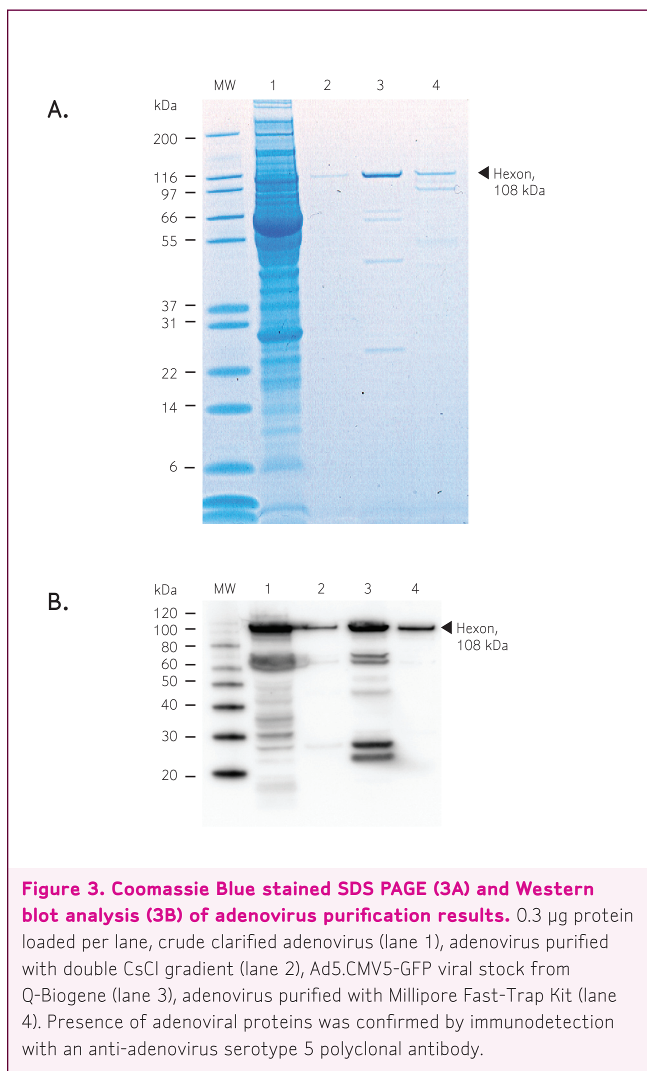

Figure 3. Coomassie Blue stained SDS PAGE (3A) and Western blot analysis (3B) of adenovirus purification results. 0.3 µg protein loaded per lane, crude clarified adenovirus (lane 1), adenovirus purified with double CsCl gradient (lane 2), Ad5.CMV5-GFP viral stock from Q-Biogene (lane 3), adenovirus purified with Millipore Fast-Trap Kit (lane 4). Presence of adenoviral proteins was confirmed by immunodetection with an anti-adenovirus serotype 5 polyclonal antibody.

Purity of the adenovirus samples was compared using SDS-PAGE (Figure 3A). This demonstrated that the Fast-Trap device (Figure 3A, lane 4) removes BSA and contaminating proteins comparable to a double CsCl purification (Figure 3A, lane 2). A Western blot with an anti-adenovirus serotype 5 polyclonal antibody (Abcam, Cat. No. 6982) confirmed that proteins detected in purified samples (Figure 3B, lane 2, and 4) are indeed adenoviral proteins.

LENTIVIRUS PURIFICATION

Crude supernatant containing VTK-945, a VSV-G pseudotyped lentivirus expressing GFP, was provided by the UNC Vector Core. The virus was purified using the components of the Millipore's Fast-Trap Lentivirus Purification and Concentration Kit.

A protocol similar to that described above for adenovirus purification was followed. The crude lentivirus supernatant was clarified using a 0.45 µm Steriflip-HV device; clarified lentivirus was mixed with 10X binding buffer and purified using a Fast-Trap Lentivirus Purification device. For further concentration and buffer exchange of the eluted purified lentivirus sample, a 100 KDa NMWL Amicon Ultra-4 filter unit was used.

To titer the different lentivirus fractions (starting material, flow-through, and purified sample), HEK293 cells were infected with serial dilutions of the virus. After three days, GFP positive cells were counted. The amounts of lentivirus bound to the membrane and recovered in elution were determined. Nearly 100% of the virus was bound, and recovery ranged from 40 to 70%. The entire workflow time including clarification, purification, and concentration/buffer exchange ranged from 45 minutes for low titer samples to 90 minutes for high titer samples (maximum capacity of Fast-Trap Lentivirus Purification filter unit is approximately 2 x 10^8 infectious viral particles). Purity was assessed by SDS-PAGE with Sypro® Ruby staining. It was confirmed that purification with the Fast-Trap device adequately removes contaminating proteins from crude lentiviral supernatant (Figure 4).

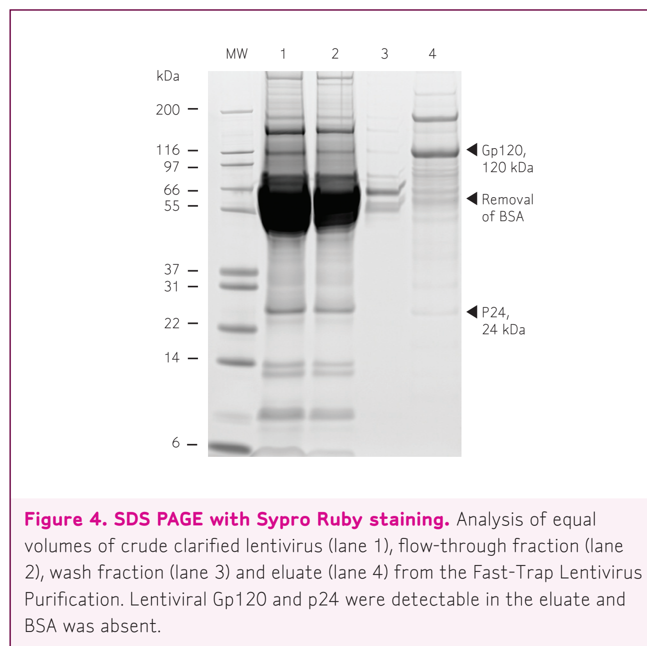

Figure 4. SDS PAGE with Sypro Ruby staining. Analysis of equal volumes of crude clarified lentivirus (lane 1), flow-through fraction (lane 2), wash fraction (lane 3) and eluate (lane 4) from the Fast-Trap Lentivirus Purification. Lentiviral Gp120 and p24 were detectable in the eluate and BSA was absent.

Performance of the Fast-Trap Lentivirus Purification and Concentration Kit was compared to the traditional method of lentiviral purification. Similar amounts of crude lentivirus were purified using the Millipore kit and a sucrose gradient ultracentrifugation (outsourced to a viral vector core facility). The recovery of the virus by sucrose gradient purification was only about 22%.

Purity of the two samples was evaluated by SDS-PAGE with Sypro Ruby staining. The load was normalized for infectious particles. Similar removal of contaminating proteins was demonstrated (Figure 5A). The presence of the 120 kDa band which corresponds to the lentiviral Gp120 glycosylated surface envelope protein in the Fast-Trap

samples (Figure 5A, lane 2 and 3) can be explained by the different mechanism of purification. Millipore Fast-Trap purification is based on binding of the viral particles onto a membrane which might explain the enrichment of surface envelope proteins in these samples. Western blot analysis

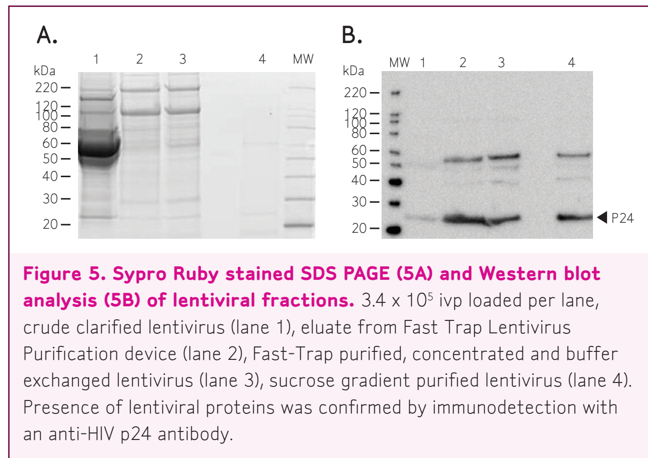

Figure 5. Sypro Ruby stained SDS PAGE (5A) and Western blot analysis (5B) of lentiviral fractions. 3.4 x 10^5 ivp loaded per lane, crude clarified lentivirus (lane 1), eluate from Fast Trap Lentivirus Purification device (lane 2), Fast-Trap purified, concentrated and buffer exchanged lentivirus (lane 3), sucrose gradient purified lentivirus (lane 4). Presence of lentiviral proteins was confirmed by immunodetection with an anti-HIV p24 antibody.

with an anti-HIV p24 antibody (Cat. No. MAB8790) confirmed that proteins detected in purified samples (Figure 5B) are indeed lentiviral proteins.

Summary

Millipore's Fast-Trap Adenovirus and Lentivirus Purification and Concentration Kits provide a fast, safe, and easy alternative for viral purification. The kits contain the necessary components to accommodate the entire virus purification workflow. The purification results in high recovery of viable viral particles with good purity.

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Progenitor Cell Targeted Culture Media Improve Primary Culture of Epithelial Cells

Jim Johnson, CELLnTEC Advanced Cell Systems, AG

Although common in many areas of research, the culture of living cells presents unique challenges to scientists. Freshly isolated primary cells most closely mimic the *in vivo* environment, but are often difficult to isolate or stop growing after only a few passages. Established cell lines can grow indefinitely, but they are transformed or derived from tumors and are therefore not accurate models of many *in vivo* processes. Also, numerous passages expose cells to selective pressures and genetic drift. This has resulted in significant experimental variances between low and high passage number cell cultures. Compounding these problems is the estimation that 18% to 36% of cell lines may be contaminated or misidentified.

The ability of CELLnTEC's Progenitor Cell Targeted (PCT) media to select and support progenitor cells now enables attractive alternatives for extended *in vitro* culture of primary cells, and also for the long-term culture of animal cells without the transformation associated with established cell lines.

Background

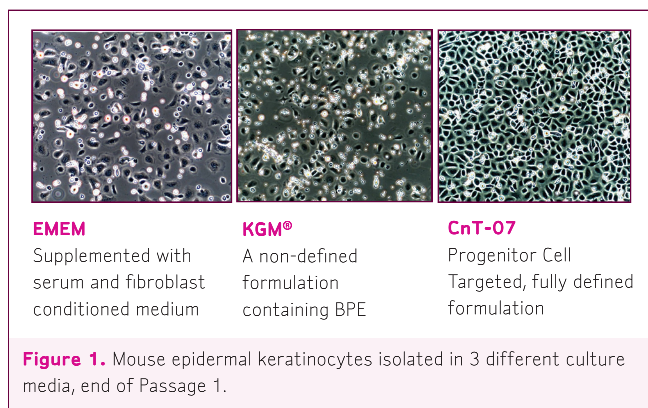
When studying living cells, selecting either the modeling accuracy of primary cells or the convenience of established cell lines can significantly affect the experimental results.

Freshly isolated cells most closely reflect the *in vivo* characteristics of normal tissues, and thus are the preferred approach for many applications. However, once the difficulties of obtaining fresh tissue plus the demands of regular isolations are factored in, many scientists often fall back on the convenience of established cell lines.

Although popular, many established cell lines have undergone various alterations, some of which may be unknown, and are therefore no longer accurate models. Established cell lines also often cause tumors when transplanted back into mice, further supporting the argument that these cell lines no longer resemble their *in vivo* counterparts in fundamental biological processes.

Likewise, the choice of defined or non-defined culture media is an example of different alternatives providing their own respective benefits and limitations. Defined media are always the preferred option, as scientists can minimize culture medium lot-to-lot variability in their experiments. However, it is well known that non-defined additives such as serum or Bovine Pituitary Extract (BPE) can improve cell growth, especially at isolation, although such undefined components introduce unknown amounts of growth factors

and cytokines. Non-defined media also tend to be less selective for the desired cell type, and often generate a culture with much more variable morphology and phenotypes (Figure 1).



EMEM
Supplemented with serum and fibroblast conditioned medium

KGM®
A non-defined formulation containing BPE

CnT-07
Progenitor Cell Targeted, fully defined formulation

Figure 1. Mouse epidermal keratinocytes isolated in 3 different culture media, end of Passage 1.

With more advanced media formulations, such as the CELLnTEC PCT media that now provide new levels of convenience and *in vivo*-like cell performance, the evaluation process of different *in vitro* tools has changed. New questions arise such as the validity of previous comparisons between primary cells and cell lines or defined and non-defined media. More recent developments and new product alternatives now shift the balance away from conclusions drawn in the past. Summarized below are a number of aspects of CELLnTEC's new PCT media which may help in the evaluation of these questions in regard to your investigations.

PCT MEDIA = MORE COLONIES, EXTENDED *IN VITRO* LIFESPAN, AND *IN VIVO*-LIKE 3D MODELS

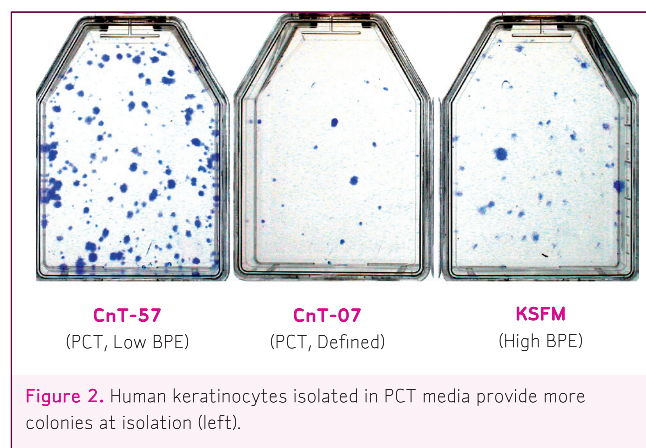
CELLnTEC PCT media offers a novel approach to epithelial media formulation by specifically targeting the self-renewing stem cells in the starting epithelial tissue. In the body, these precious progenitor cells are ideal candidates for cell culture due to their ability for long-term growth without transformation and their capacity to form many different tissue types during differentiation.

Progenitor Cell Targeted (PCT) media are a new generation of epithelial media formulated to target and retain progenitor cells. Based on the signaling pathways involved in the adult stem cell microenvironment, this media enhances isolation of the progenitor cell population and encourages the growth of undifferentiated progenitor cells while retaining their *in vivo* like characteristics. In comparison, traditional, older media formulations were built almost exclusively on nutrient profiles which did not consider the need of cells for a specific signaling environment.

Cells isolated and grown in PCT media have been found to develop more colonies and to have an extended *in vitro* lifespan (Figure 2), all while using vastly simplified protocols. This longevity allows more cells to be grown, increasing the number of experiments per isolation while minimizing culture variability and the number of new isolations required. These progenitor cells may also be induced to differentiate normally for adhesion studies and the establishment of 3D models, and can be easily switched from our special low-BPE media to fully defined media as required. Primary epithelial cells are available from epidermis, bladder, and cornea (see full list on following pages).

Cells isolated in PCT media:

- Develop more colonies
- Have an extended *in vitro* lifespan
- Are accurate models of the *in vivo* situation



CnT-57
(PCT, Low BPE)

CnT-07
(PCT, Defined)

KSFM
(High BPE)

Figure 2. Human keratinocytes isolated in PCT media provide more colonies at isolation (left).

LONG-TERM ANIMAL CELL CULTURE WITHOUT TRANSFORMATION

PCT media have also opened up a new alternative for long-term culture without the need for active transformation or the use of tumorigenic tissue. Millipore now offers CELLnTEC long-term guaranteed cell systems. Isolated from normal tissue using CELLnTEC medium, these cells have not been transfected to provide indefinite growth, unlike many conventional cell lines.

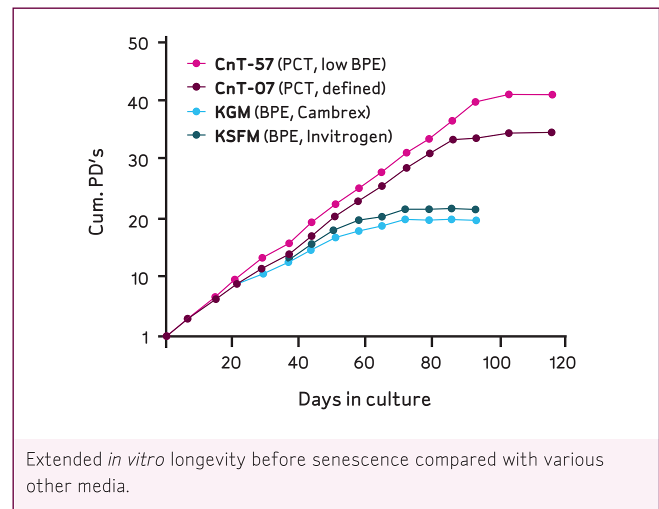
These long-term cell systems are supplied at approximately passage 25, and are guaranteed to provide at least 6 months (~ 25 more passages) of additional growth in your lab. Routinely, these cells grow for more than 50 passages, during which they have been found to stably express a range of markers. Also, they do not cause tumors when re-implanted *in vivo*.

Cells are available for various tissues (epidermis, airway, prostate, bladder) from several animal species (mouse, rat, rabbit – see list below) and represent an *in vitro* tool, providing stable, dependable modeling for your research in the long term.

EXPAND YOUR *IN VITRO* OPTIONS WITH CELLS GROWN IN PCT MEDIA

As outlined above, PCT media bring colony formation and longevity benefits to primary epithelial cell culture and have also been used to generate long-term cultures, creating a valuable alternative to transformed cell lines.

In addition to guaranteed primary and long-term cell cultures, CELLnTEC also provides a complete range of isolation, culture, transfection, and differentiation protocols to ensure maximum ease and efficiency in all your *in vitro* procedures. For more details, please visit the resources section on **www.cellntec.com**.



Extended *in vitro* longevity before senescence compared with various other media.

Human Cells

Description	Frozen vial	Vial & Media Kit	Catalogue No.
Bladder Epithelium Progenitors, human	1 x >5 x 10^5 Cells	Kit	#HBEP.05
Corneal Epithelium Progenitors, human	1 x >5 x 10^5 Cells	Kit	#HCEP.05
Epidermal Keratinocyte Progenitors, pooled, human	1 x >5 x 10^5 Cells	Kit	#HPEKp.05
Epidermal Keratinocyte Progenitors, pooled, human	3 x >5 x 10^5 Cells	Kit	#HPEKp.15
Epidermal Keratinocyte Progenitors, single donor, human	1 x >5 x 10^5 Cells	Kit	#HPEKs.05
Epidermal Keratinocyte Progenitors, single donor, human	3 x >5 x 10^5 Cells	Kit	#HPEKs.15
Dermal Fibroblasts, human	1 x >5 x 10^5 Cells	Kit	#HDFS-05
Coming Soon! Prostate Epithelium Progenitors, human	1 x >5 x 10^5 Cells	Kit	#HPEP.05
Coming Soon! Prostate Epithelium Progenitors, human	3 x >5 x 10^5 Cells	Kit	#HPEP.15

Other Animal Cells

Description	Frozen vial	Vial & Media Kit	Catalogue No.
Epidermal Keratinocyte Progenitors, C57BL/6, mouse	1 x >6.5 x 10^5 Cells	Kit	MPEK-BL6
Epidermal Keratinocyte Progenitors, Rosa, mouse	1 x >6.5 x 10^5 Cells	Kit	MPEK-ROSA
Epidermal Keratinocyte Progenitors, 129, mouse	1 x >6.5 x 10^5 Cells	Kit	MPEK-129
Epidermal Keratinocyte Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RPEK-WIS
Epidermal Keratinocyte Progenitors, Sprague Dawley, rat	1 x >6.5 x 10^5 Cells	Kit	RPEK-SD
Epidermal Keratinocyte Progenitors, Beagle, dog	1 x >6.5 x 10^5 Cells	Kit	CPEK
Dermal Fibroblasts, BalbC, mouse	1 x >6.5 x 10^5 Cells	Kit	DF-BALBC
Dermal Fibroblasts, rat	1 x >6.5 x 10^5 Cells	Kit	DF-R
Dermal Fibroblasts, rabbit	1 x >6.5 x 10^5 Cells	Kit	DF-B
Vaginal Keratinocyte Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RPVAK-WIS
Vaginal Keratinocyte Progenitors, rabbit	1 x >6.5 x 10^5 Cells	Kit	BPVAK
Small Airway Epithelium Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RSAK-WIS
Small Airway Epithelium Progenitors, Sprague Dawley, rat	1 x >6.5 x 10^5 Cells	Kit	RSAK-SD
Large Airway Epithelium Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RLAK-WIS
Large Airway Epithelium Progenitors, Sprague Dawley, rat	1 x >6.5 x 10^5 Cells	Kit	RLAK-SD
Bladder Urothelium Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RBLAK-WIS
Bladder Urothelium Progenitors, Sprague Dawley, rat	1 x >6.5 x 10^5 Cells	Kit	RBLAK-SD
Prostate Epithelium Progenitors, Wistar, rat	1 x >6.5 x 10^5 Cells	Kit	RPROK-WIS
Prostate Epithelium Progenitors, Sprague Dawley, rat	1 x >6.5 x 10^5 Cells	Kit	RPROK-SD

Summary

CELLnTEC Progenitor Cell Targeted media, along with our extensively tested protocols, now enable new levels of convenience and cell performance in both primary and long-term culture. In light of these new products and capabilities, researchers may now more easily make the transition from cell lines to primary culture, or from normal tissue to long-term cell cultures. These media and cell products are easily combined with a range of related antibiotic and enzyme products, plus Millicell® inserts for 3D culture. Collectively, these synergistic product lines represent a new level in cell culture performance and convenience. Free media samples are available; please contact your local sales representative to coordinate.

Related Products

Description	Frozen vial	Catalogue No.
Antibiotic/Antimycotic Solution (100X)	100 mL	CnT-ABM
Antibiotic/Antimycotic Solution (200X, ready to use single aliquots)	10 x 2.5 mL	CnT-ABM10
Antibiotic/Antimycotic Solution (200X, ready to use single aliquots)	20 x 2.5 mL	CnT-ABM20
Neutral Protease (Dispase), functionally tested	1 gm	CnT-NPD-01

ENStem-A Adherent Human Neural Progenitors: A New Source of Primary Human Neural Cells

Dave Machacek, Kate Hodges, Laurie Murrah-Hanson, Sujoy K. Dhara, Brian A. Gerwe, Kowser Hasneen, Mahesh C. Dodla, Carla D. Sturkie, and Steve Stice. Aruna Biomedical and Univ of Georgia.

Abstract

Progenitor cell populations derived from human embryonic stem cells (hESCs) can serve as a renewable source of physiologically relevant human primary neural cells for basic research and drug discovery. Repeated derivation of neural progenitors from hESCs can lead to increased variability, along with the difficulty and expense of hESC culture. Millipore's new ENStem-A cells offer an attractive alternative: they proliferate as an adherent monolayer in serum-free, feeder-free conditions. Here we discuss the characteristics and functional assays in which ENStem-A cells can be used.

Introduction

Most neurons are produced during embryonic development by neural stem cells or neural progenitor cells. A new method to derive neural progenitors from NIH-approved human embryonic stem cells (hESCs)[1], has been licensed by Aruna Biomedical Inc. from the University of Georgia. This novel method allowed the development of a convenient, ready-to-use kit containing cryopreserved neural progenitors and the reagents required to propagate and differentiate them.

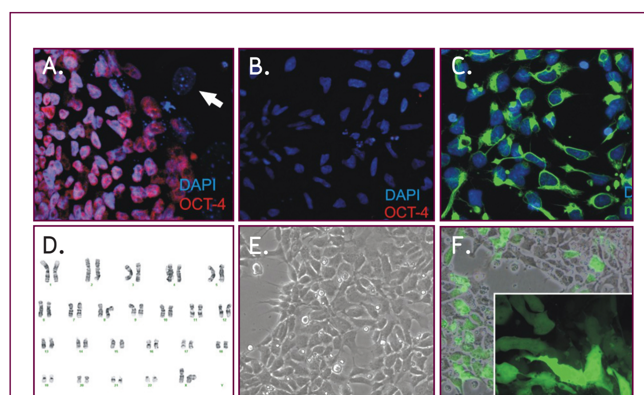


Figure 1. ENStem-A cells demonstrate the expected immunoreactivity, chromosome number, and transduction capacity. (A) WA09 human ESC are Oct-4 positive, while mouse feeder cells are negative (arrows). (B) ENStem-A cells are Oct-4-negative. (C) ENStem-A cells labeled for nestin immunoreactivity and display normal karyotype (D) and genetics consistent with their WA09 origin. (E) Phase contrast image of progenitors growing in an adherent monolayer. (F) Cells can easily be transduced with reporter genes demonstrating cells that are expressing GFP after lenti-viral transduction.

The cells and kit are produced by Aruna Biomedical and distributed by Millipore under the ENStem-A brand name.

Aside from their convenience, ENStem-A cells possess many useful properties. They are not transformed, making them particularly interesting for studying cell cycle regulation and proliferation because pathways involving histone acetyltransferases have not been altered or over expressed. Additionally, since the cells were derived from a registered stem cell line (WA09), the genetic background of the original donor is well documented. These cells have a normal compliment of chromosomes (euploidy) and are preserved without any genetic modifications to alter endogenous signaling pathways (Figure 1). Due to their proliferative capacity, they are amenable to genetic manipulations if this is desired by the end-user (Figure 1). ENStem-A cells have been shown to differentiate into multiple neural phenotypes including glia and motoneurons[1,2]. Use of growth factors and/or genetic markers will no doubt be useful for enrichment of neural cell phenotypes. They also do not express high levels of pluripotent markers like Oct-4 and are nestin positive.

Methods and Results

DRUG DISCOVERY

Though we have routinely grown ENStem-A cells in a variety of formats ranging from 35 mm dishes to T-300 flasks on poly-ornithine and laminin coated plates, we have recently demonstrated that they can also be grown in 96-well plates. Substrates tested for this format include collagen IV, fibronectin, and vitronectin, offering greater assay versatility (Figure 2). Differentiating these cells using our optimized ENStem-A differentiation media results in β-III tubulin positive cells which can be plated into 96-well plates and assayed. We have demonstrated the presence of functional AMPA receptors on these differentiated populations by using a Flex Station Fluorometer (Molecular Devices) to record dose-dependent changes in internal calcium concentration in response to AMPA (Figure 3).

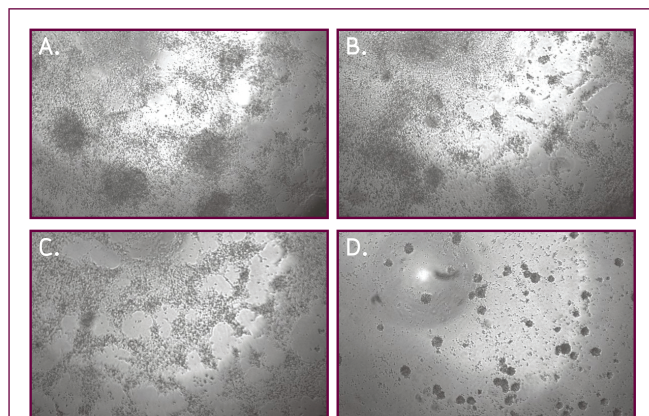


Figure 2. ENStem-A cells can be grown in 96 well plates on a variety of substrates. Phase contrast images at using 4x objective growing ENStem-A cells on collagen IV (A), fibronectin (B) and vitronectin (C). Cells plated without substrate on BSA treated wells did not grow plate efficiently.

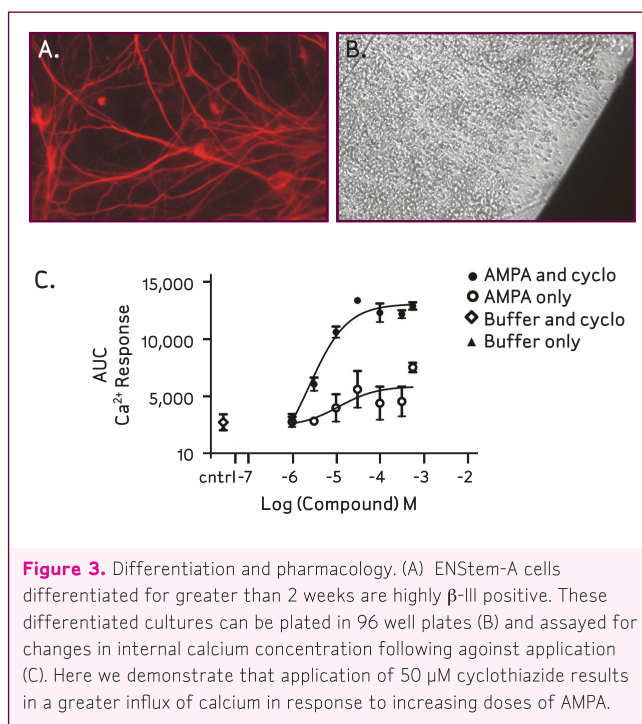


Figure 3. Differentiation and pharmacology. (A) ENStem-A cells differentiated for greater than 2 weeks are highly β-III positive. These differentiated cultures can be plated in 96 well plates (B) and assayed for changes in internal calcium concentration following agonist application (C). Here we demonstrate that application of 50 μM cyclothiazide results in a greater influx of calcium in response to increasing doses of AMPA.

GENETIC MODIFICATIONS

Our recently published work on developing a drug screening assay for spinal muscular atrophy demonstrates that reporter genes can be introduced and expressed in ENStem-A cells[3]. This preliminary work may lead to new assays for other neuronal diseases and developmental neurotoxicity. Genetic reporters also help elucidate cellular function and track molecular pathways involved in development and differentiation.

We have also tested several means of transfecting and transducing ENStem-A cells. All lines were maintained on poly-ornithine laminin per our optimized protocols. To understand how efficiently these cells can be genetically manipulated, we used three very different systems (eletroporation by Nucleofector$_{[MC1]}$, transfection by ExGen500$_{[MC2]}$ and transduction by a lentiviral system) to deliver a green fluorescent protein (GFP) sequence under the control of the murine ubiquitous promoter, Ubiquitin C. To quantify the efficiency of delivery, we measured GFP expression by flow cytometry after 72 hours. At this time the number of live and dead cells were determined using propidium iodide. Transduced cells were further propagated to determine expression levels over time.

Based on percent GFP+ live cells, transduction by the lentiviral system was the most efficient. 35% of transfected ENStem-A cells expressed GFP+ and could be further purified using FAC >90% (Figure 4); transfection with ExGen500 (MBI Fermentas) was the least efficient with less than 3% GFP+ cells, and nucleofection gave mid-range results of 16% GFP+ cells. We also evaluated cell death with these three delivery techniques. The ENStem-A cells tolerated lentiviral transduction (~40% survival) more readily than the other techniques which had survival rates of 6% and 13% for nucleofection and ExGen 500, respectively. We continued culturing transduced ENStem-A cells for >15 passages to show that they retained their differentiation potential. ENStem-A cells can be modified using various techniques; however, when considering three parameters (% initial GFP+, viability and long term GFP expression), the lentiviral method performed better than other gene insertion methods tested.

Conclusion

ENStem-A cell monolayer cultures are uniform, robust, easily propagated, and capable of differentiating into many neural lineages. They grow on multiple substrates, can be genetically modified, and develop functional receptors which can be screened in a 96 well format. Thus, these cells are useful for many assays including neuroprotection, neurite outgrowth, synaptogenesis, growth cone collapse, genotoxicity, cell cycle regulation, DNA repair, cellular pathways (phenotypic differentiation, intracellular signaling), and pharmacology (dose response relationships, receptor desensitization).

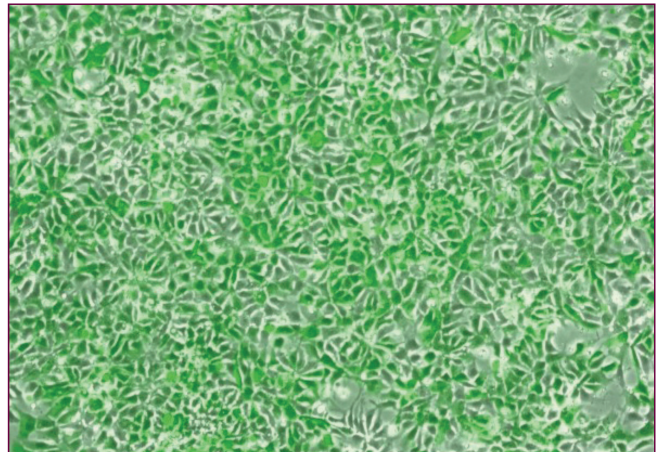


Figure 4. Lentiviral transduction of constitutive GFP expression. A phase-contrast superimposition onto GFP fluorescence demonstrates that roughly 90% of cells are transduced in initial experiments. Image courtesy of Aruna Biomedical.

Millipore Products

Description	Cat. No.
ENStem-A Human Neural Progenitor Expansion Kit	SCR055
ENStem-A Neural Freezing Medium, 50 mL	SCM011
ENStem-A Neural Expansion Medium, 500 mL	SCM004
ENStem-A Neuronal Differentiation Medium, 100 mL	SCM017

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Embryoid Body Differentiation Methods for Neurogenesis and Adipogenesis

Nick Asbrock, Amy Botica, and Vi Chu, Ph.D., Millipore Corporation, Temecula, CA

Abstract

The study of stem cell differentiation provides a wealth of information about the developmental process of many types of cells, including neurons and adipocytes. Most protocols used to differentiate murine embryonic stem cells involve the formation of an embryoid body, but the exact protocols vary. Here we report on the efficacy of a new Embryoid Body Formation Medium to aid in the differentiation of mouse embryonic stem cells to neurons and adipocytes.

Introduction

Current *in vitro* assays of neurogenesis and adipogenesis rely on cells obtained from a number of different sources. These include freshly isolated tissues, neural or pre-adipocyte cell lines such as PC-12 and 3T3-L1, and tissue-specific stem cells, including neural and mesenchymal stem cells, respectively. Because of their robust growth characteristics and pluripotent nature, embryonic stem (ES) cells have also emerged as an attractive model for studying the earliest stages of development and lineage specificity.

Many embryonic stem cell differentiation protocols rely on the spontaneous differentiation that occurs within 3-dimensional aggregates of cells termed embryoid bodies (EBs). EBs are spontaneously formed when embryonic stem (ES) cells are suspension-cultured in low attachment Petri dishes. It is thought that EB formation mimics the earliest stages of embryonic development, making for a much more accurate model of *in vivo* differentiation.

To facilitate the formation of EBs for ES cell differentiation, we have developed and optimized an EB Formation Medium. The medium can be used to form EBs in hanging drops or suspension culture on non-adhesive Petri dishes. In addition, we have designed and validated specific assays using this medium to preferentially differentiate mouse ES cells into neurons and adipocytes with specific inducers.

Methods

Mouse ES cells were cultured in normal ES growth medium containing 15% serum and LIF on 0.1% gelatin coated 10 cm tissue culture plates at 37 °C and 9-10% CO_2. When 80% confluent, the cells were incubated with 5 mL of Accutase™ cell dissociation solution for 3-5 minutes in a 37 °C incubator to detach the cells. Once cells were completely detached, 5 mL of the EB Formation Medium (pre-warmed to 37 °C) was added to the plate. The cells were then centrifuged at 300 xg for 2-3 minutes, the supernatant was discarded, and the pelleted cells were resuspended in 2 mL of EB Formation Medium.

To develop a EB suspension culture, 2 to 3 x 10^6 cells were aliquoted in 10 mL EB Formation Medium and placed in a sterile 10-cm bacterial ultra low attachment Petri dish. Cells were incubated in a 37 °C, 9-10% CO_2 incubator for two to four days depending on the differentiation protocol. Using a 10 mL serological pipette, cells (both aggregated EBs and non aggregated cells) were carefully collected into a 50 mL conical tube and allowed to gravity pellet for 15 minutes.

For induction of adipogenesis, EBs were collected and incubated overnight in a solution of 10 mL EB Formation Medium and 2 µL of 500 µM retinoic acid. This process was repeated with fresh media each day for 3 days. EBs were then further differentiated by seeding 10-20 EBs/well on a 24-well gelatin-coated plate and incubating in Differentiation Medium (EB Formation Medium, 20 nM T3 (3,3′,5-triiodo-L-thyronine) and 850 nM insulin). Differentiation media was changed every two days for 21 days, with adipocyte-like cells becoming visible around day 14. Staining and quantification with Oil Red O confirmed the identity of lipid-containing adipocytes.

For induction of neurogenesis, EBs were collected and incubated for two days in a solution of 10 mL EB Formation Medium and 2 µL of 500 µM retinoic acid. This process was repeated with fresh media every other day for a total of four days. EBs were then expanded by seeding 10-20 EBs/well of an eight-well laminin/poly-L-ornithine-coated glass chamber slide and incubating in EB Formation Medium. Media was changed every two days for eight days total, with neuronal-like cells appearing in as little as four days. Cells were then fixed in 4% PFA, blocked in 5% normal donkey serum, and immunofluorescently labeled with a mouse anti-βIII-tubulin antibody and DAPI counterstain to confirm the neuronal identity of the cells.

Results and Discussion

When used for adipogenesis or neurogenesis, the EB Formation Medium performed as expected, helping to create and sustain EBs in suspension culture (Figure 1A). The addition of retinoic acid to the media was necessary to initiate differentiation into either adipocytes or neurons (Figure 1B).

To further direct cells toward adipocyte lineages, the subsequent addition of insulin and T3 was required. Staining with Oil Red O after 21 days clearly demonstrated a marked increase in the number of adipocytes developed with the EB media (Figure 2A) as compared to cells that were allowed to spontaneously differentiate (Figure 2B).

In the case of neuronal differentiation, we found that more than 50% of EBs contained βIII-tubulin positive neurons that emanated from the densely packed EBs (Figure 3A). Overall, approximately 20-30% of all the cells in the culture stained positive for βIII-tubulin (data not shown). We hypothesize that the limited differentiation observed was due to the spherical conformation of EBs, which inherently prevents cells within the EB from accessing the same amount of retinoic acid as cells on the outside of the EB. Nonetheless, we find that the EB differentiation method provides a good model system for neuronal differentiation as the fully differentiated cells outside the EB appear mature, forming complex neural networks with large neurites and extensive branching (Figure 3B).

The EB Formation Medium was shown to play a critical role in supporting the formation of EBs and in assisting with the directed differentiation of murine ESCs down both neuronal and adipogenic lineages. This medium is available as a stand alone product and in differentiation kits for neurogenesis and adipogenesis. Our kits include the all the reagents needed to follow our differentiation protocols, such as EB medium, differentiation inducers and growth factors, Accutase solution, ECMs, and dye/antibodies for cell lineage detection. We also offer the EB Formation Medium separately for researchers interested in exploring different protocols. Our novel media formulation offers both a validated protocol and greater flexibility for all researchers using EBs to differentiate ESCs, whether for adipogenesis or neurogenesis.

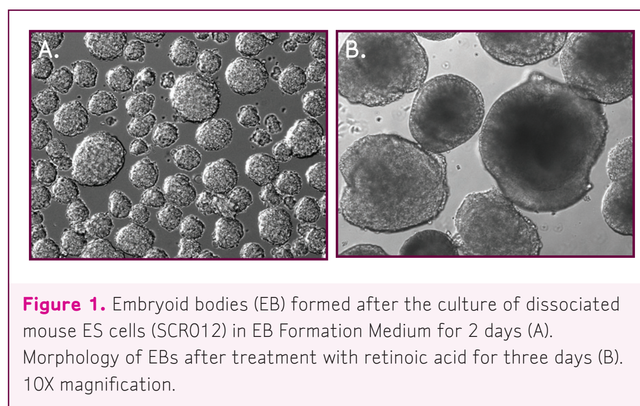

Figure 1. Embryoid bodies (EB) formed after the culture of dissociated mouse ES cells (SCR012) in EB Formation Medium for 2 days (A). Morphology of EBs after treatment with retinoic acid for three days (B). 10X magnification.

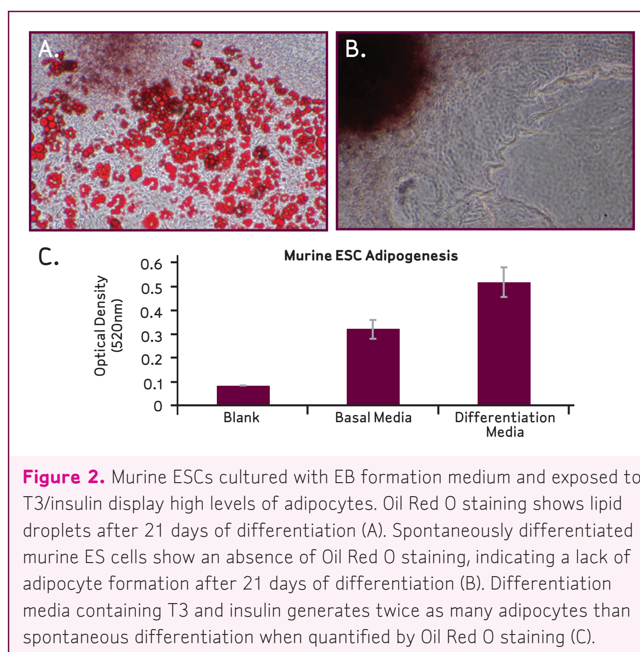

Figure 2. Murine ESCs cultured with EB formation medium and exposed to T3/insulin display high levels of adipocytes. Oil Red O staining shows lipid droplets after 21 days of differentiation (A). Spontaneously differentiated murine ES cells show an absence of Oil Red O staining, indicating a lack of adipocyte formation after 21 days of differentiation (B). Differentiation media containing T3 and insulin generates twice as many adipocytes than spontaneous differentiation when quantified by Oil Red O staining (C).

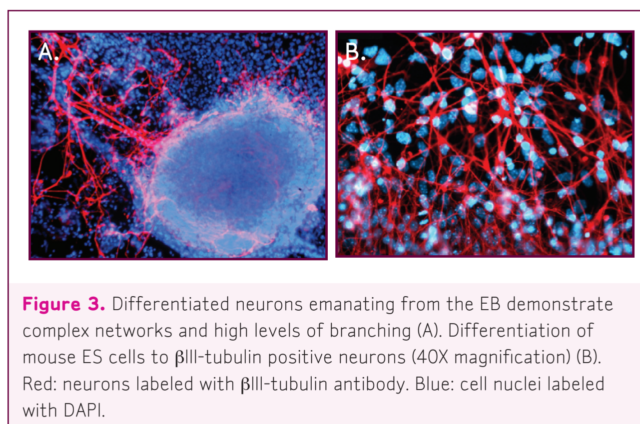

Figure 3. Differentiated neurons emanating from the EB demonstrate complex networks and high levels of branching (A). Differentiation of mouse ES cells to βIII-tubulin positive neurons (40X magnification) (B). Red: neurons labeled with βIII-tubulin antibody. Blue: cell nuclei labeled with DAPI.

Millipore Products

Description	Cat. No.
Mouse Embryonic Stem Cell Adipogenesis Kit	SCR100
Mouse Embryonic Stem Cell Neurogenesis Kit	SCR101
Embryoid Body (EB) Formation Medium	SCM018
Accutase Cell Dissociation Solution	SCR005
Pluristem 126/S6 Murine ES cells	SCR012

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New in Apoptosis Imaging: Dual Detection of Self-Execution and Waste-Management

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Abstract

Apoptosis enables the orderly disposal of unwanted cells without an inflammatory response. In the process, DNA fragments with defined ends are created, a phenomenon used by many commercially available apoptosis detection kits. While many kits are very sensitive and versatile, all have a major drawback: they are only able to detect the presence of DNA fragments with DNase I type ends, 3′OH/5′PO_4. However, apoptosis also generates fragments with DNase II type ends, 3′PO_4/5′OH (Figure 1). Here we report a new apoptosis detection method which enables researchers to detect both types of DNA fragments simultaneously in a single sample.

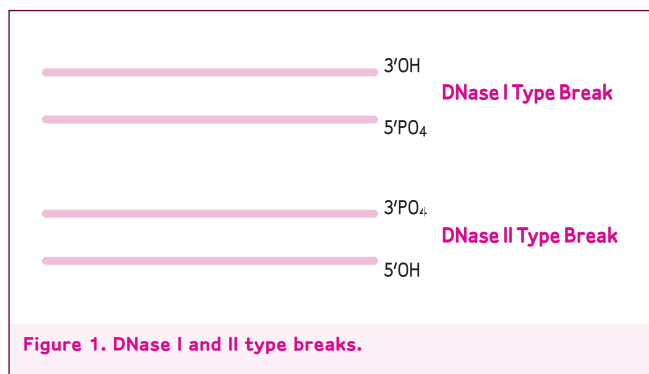


Figure 1. DNase I and II type breaks.

Introduction

During apoptosis, cells undergo sequential steps leading to their complete disassembly and disappearance. Massive and systematic DNA fragmentation is a characteristic feature of this process. Not surprisingly, these DNA fragments are often used as specific markers in apoptosis detection. DNA fragments created in apoptosis are not random. Usually, they possess blunt ends or short, single-nucleotide staggers[1, 2]. A double-strand (ds) break forms when a DNA duplex is cut through, exposing the 3′ and 5′ ends of the two DNA strands. These ends can carry either a phosphate (PO_4) group or a hydroxyl (OH) group (Figure 1). The distribution of these groups provides important information about the enzyme that cut the DNA.

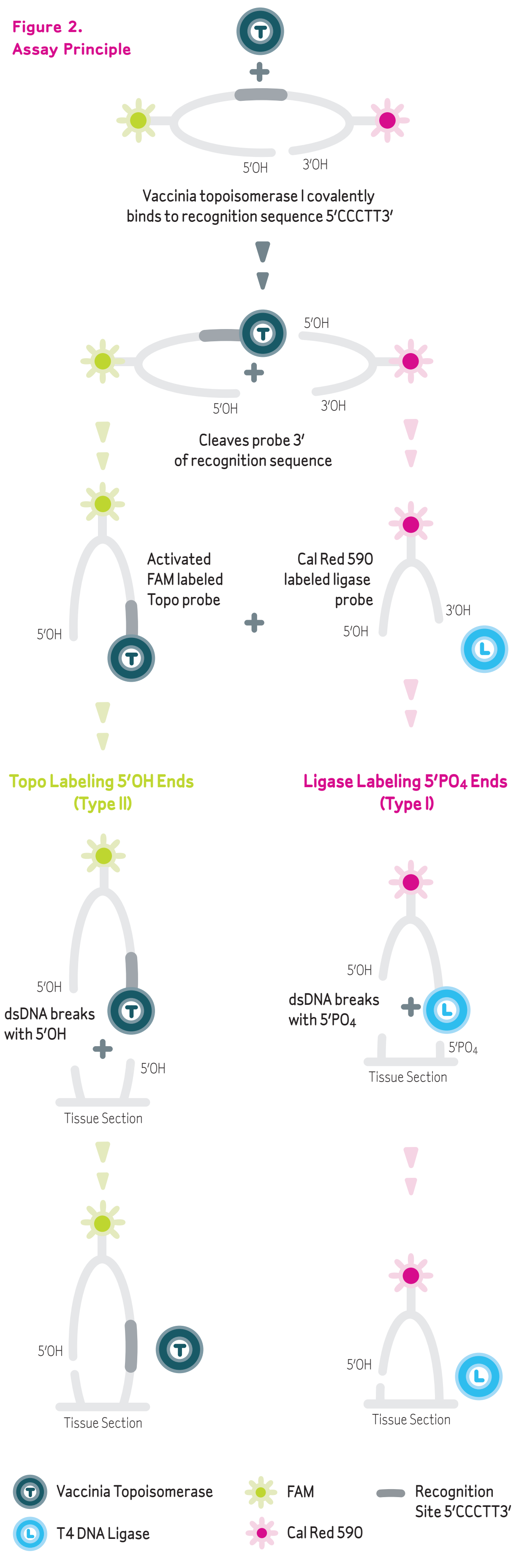
Cuts with a 3′OH/5′PO_4 configuration are termed DNase I type cuts. Cuts with the opposite configuration, 3′PO_4/5′OH, are called DNase II type[3, 4]. These cuts received their names because they match the cleavage patterns of the two major nucleases, DNase I and DNase II. These names are used for convenience – the actual apoptotic nuclease involved may or may not be related to DNase I and II.

Different properties of DNase I cleavage are used by various apoptosis detection assays. For example, several of Millipore's ApopTag® kits specifically detect one marker of DNase I cleavage by labeling the 3′OH groups with help of the terminal deoxyribonucleotidyl transferase (TdT) enzyme. Millipore's related assay, the ApopTag Oligo Ligation kit, detects three parameters at once by using T4DNA ligase and is even more specific. Although highly sensitive, both of these assays detect only DNase I type fragments—leaving cells with DNase II type cleavage undetected. Until recently, there were no methods that could specifically detect DNase II type breaks.

New developments in apoptosis research have changed the very concept of apoptotic cell elimination and significantly increased the value of detecting DNase II type breaks. Apoptosis is no longer viewed as an individual cellular event. The complete apoptotic process is now thought to include two phases: self-execution and externally-controlled elimination of apoptotic cell corpses by waste-management cells[7]. The externalized waste-control phase is essential, highly conserved, and considered to be even more important than the internal phase of cell disassembly[5,6,7], because it ensures the complete degradation of the dying cell's DNA. This prevents release of pathological, viral, and tumor DNA and self-immunization.

DNase II plays a fundamental role in the waste-management phase of apoptosis[5,8]. DNase II is present in lysosomes, the sac-like organelles that contain digestive enzymes used in breaking down cellular components. The enzyme destroys the DNA of apoptotic cells after their corpses are engulfed by tissue macrophages and other waste-management cells.

The new ApopTag ISOL Dual Fluorescence Apoptosis Detection kit addresses these important developments[9].



It offers new dual-detection technology which labels both the self-execution and waste-management phases of apoptotic DNA degradation.

Methods

This new technique uses a novel oligonucleotide probe for detection[9]. This self-annealing oligo contains two complementary base sequences that spontaneously form a dual hairpin secondary structure (Figure 2). The oligo also has two fluorescent labels (FAM and Cal Red 590) at opposite poles of the dual hairpin.

Topoisomerase I cuts the DNA at the 3′ end of the recognition site, splitting the dual hairpin oligo into two differently-labeled hairpin oligonucleotides. The biochemical specificity of the provided enzymes impacts the detection aspect of the protocol, in that vaccinia topoisomerase I will recognize and ligate the FAM oligo to 5′ OH DNA ends (DNase type II cuts) whereas T4 DNA Ligase will recognize and ligate the CR590 labeled oligo to 5′ PO_4 DNA ends (DNase type I cuts).

The actual staining protocol is quite simple, and works with a variety of sample types including paraffin-embedded tissue, frozen tissue sections, cell suspensions, and adherent cells. Samples are fixed and prepared for staining according to their type, incubated for 10-16 hours with the dual labeling solution, washed, counterstained, and visualized using fluorescence microscopy.

Results & Discussion

Unlike its predecessors, the ApopTag ISOL Dual Fluorescence Apoptosis Detection kit specifically detects both DNase I and II type breaks. Both vaccinia topoisomerase I and T4 DNA ligase labeled only one type of DNA break each when tested in bovine tissue (Figure 3).

Tests in rat thymus showed the full capacity of this new kit. Cortical macrophages with engulfed nuclear material and apoptotic thymocytes were clearly labeled. Cytoplasmic

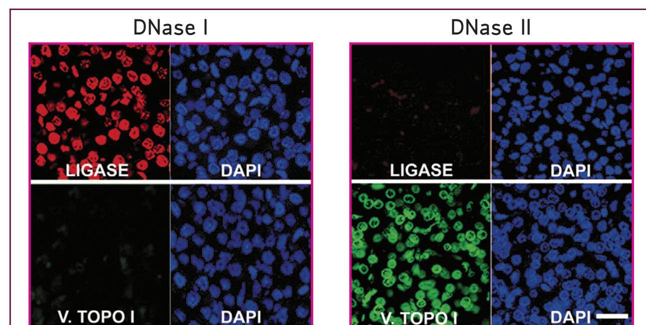


Figure 3. Selective detection of two major types of DNA damage. Sections of normal bovine adrenal tissue were treated with either DNase I to produce 3′OH/5′PO_4 blunt-ended breaks or DNase II to produce 3′ PO_4/5′ OH blunt-ended breaks. Bar - 25 μm.

fluorescence revealed lysosomes containing DNA with 5′OH double-strand breaks. Surrounding thymocytes underwent apoptosis and had 5′PO_4 double-strand breaks located at the periphery of their nuclei (Figure 4). Both phases are also seen in the lower magnification view of apoptotic thymus tissue in Figure 5.

Through the use of novel oligonucleotide probes, the ApopTag ISOL Dual Fluorescence Apoptosis Detection kit overcomes the disadvantages of traditional apoptosis detection kits. This new kit labels both DNase I and DNase II-type apoptotic cleavage, expanding the range of detectable DNA breaks and addressing the wider impact of apoptosis in tissue. The dual-detection format enables scientists to clearly visualize both the self-execution and waste-management phases of apoptosis in a single sample, making research faster and more convenient than ever before.

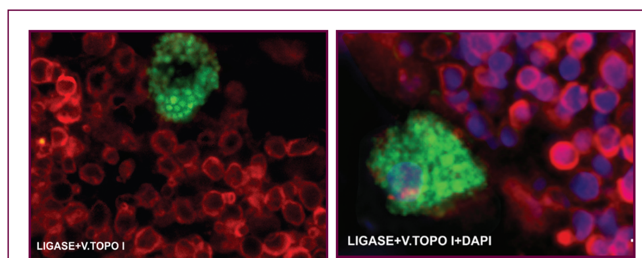


Figure 4. Dual detection of the execution and waste-management phases of apoptosis. Dexamethazone-treated apoptotic rat thymus. Red: ligase-based detection of self-execution phase, green: topoisomerase-based detection of waste-management phase, blue: DAPI nuclear stain. Bar - 15 μm.

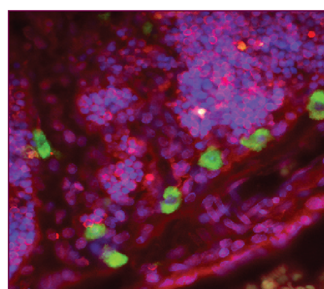


Figure 5. Macrophages loaded with apoptotic DNA in the waste-management phase of apoptosis in thymus. Green: macrophages with engulfed apoptotic corpses (TOPO labeling), red: apoptotic thymocytes (*in situ* ligation), blue: nuclear staining (DAPI).

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Millipore Products

Description	Cat. No.
ApopTag ISOL Dual Fluorescence Apoptosis Detection Kit (DNase Types I & II)	APT1000
ANTI-FAS Monoclonal	05-201
Anti-Caspase 3, active (cleaved) form	AB3623
Anti-Bak, NT	06-536
Anti-Bax, NT	06-499
Anti-Clusterin α chain (human), clone 41D	05-354
Anti-Cyclophilin A	07-313
Anti-Caspase 1	06-503
Anti-DNA, single stranded specific, clone F7-26	MAB3299
Anti-Fas (human, neutralizing), clone ZB4	05-338
Anti-Phosphatidylserine, clone 1H6, Alexa Fluor 488 conjugate	16-256
Anti-Phosphatidylserine, clone 1H6	05-719
Anti-Bcl2, clone 100	05-729
Anti-Cystatin C	06-458
Anti-Caspase 3, large subunit & proform	AB1899
Anti-Bim, internal epitope, pan-Bim isoforms	AB17003
Anti-AIF, internal domain	AB16501
Anti-Cathepsin D	06-467
Anti-Bim, clone 14A8	MAB17001-50UG
Anti-Caspase 8	AB1879
Anti-Caspase 3	06-735
Anti-Caspase 9, clone 96-2-22	05-572
Anti-FADD, clone 1F7	05-486
Anti-Poly ADP-ribose, clone 10H	MAB3192
Anti-Cathepsin B	06-480

New Products

HEScGRO™ Basal Medium

HEScGRO Basal hES Medium is a basal formulation of HEScGRO medium (without bFGF) that is specifically created to meet the special requirements of human embryonic stem cell culture. This medium is defined, serum-free, and animal-component-free. Unlike the complete HEScGRO Medium, HEScGRO Basal Medium does not contain bFGF, thereby allowing flexibility of growth factor component formulation, yet still providing the benefits of an animal-component-free, serum-free medium.

Description	Qty/Pk	Cat. No.
HEScGRO Basal Medium for Human ES Cell Culture	5 x 100 mL aliquots	SCM021

Human Embryoid Body Formation Medium

Human Embryonic Stem (ES) Cell Embryoid Body Formation Medium is designed to support the spontaneous or directed differentiation of human embryonic stem cells. The medium can be used to form embryoid bodies in suspension culture on low adhesion plates. Embryoid bodies formed using SCM026 have been shown to facilitate the differentiation of human ES cells into neural, endodermal, and cardiac cell lineages.

Description	Qty/Pk	Cat. No.
Human ES Embryoid Body Formation Medium	5 x 100 mL aliquots	SCM026

CELLnTEC Media for Epithelial Cell Culture

CELLnTEC Media improve isolation and growth of multiple types of epithelia. This comprehensive range of optimized media includes unique, revolutionary Progenitor Cell Targeted (PCT) Media for serum-free and feeder-free isolation and expansion of epithelial progenitors. Millipore has recently added new kit formats to the extensive CELLnTEC range.

Description	Qty/Pk	Cat. No.
PCT Epidermal Keratinocyte Medium, Low BPE	500 mL	CnT-57
PCT Epidermal Keratinocyte Medium, Low BPE, Calcium Free	500 mL	CnT-57CF
PCT Bladder Epithelium Medium, Low BPE	500 mL	CnT-58

EmbryoMax® ES Cell Qualified Fetal Bovine Serum, New Zealand Origin

The EmbryoMax ES Cell Qualified FBS from New Zealand (lot number 060102) is Millipore's newest addition to our comprehensive range of ES cell qualified products. This lot of serum has been selected for its exceptional ability to maintain the undifferentiated state of mouse ES cells in standard culture conditions. The use of serum pre-qualified for mouse ES cell culture applications eliminates the need for stem cell researchers to screen multiple lots of serum, thus delivering significant cost and time savings.

Description	Qty/Pk	Cat. No.
EmbryoMax ES Cell Qualified FBS, New Zealand origin	500 mL	ES-011-B

Bone Marrow Harvesting and HSC Isolation Kit

The Bone Marrow Harvesting and HSC Isolation Kit uses a combination of mechanical fragmentation and enzymatic digestion to isolate bone marrow cells and HSCs from murine bone tissue, and, uniquely, from the endosteum. This is especially helpful, as research demonstrates that this method results in up to a six-fold greater recovery of primitive hematopoietic cells (lineage- Sca-1+ c-kit+ [LSK] cells) and HSCs as shown by transplant studies[1].

Description	Qty/Pk	Cat. No.
Bone Marrow Harvesting and HSC Isolation Kit	10 isolations	SCR051

Human Epithelial Progenitor Cells

Millipore now offers numerous human progenitor cell types as monolayer cell systems, including Human Epidermal Keratinocytes (from neonatal foreskins), human bladder epithelial progenitors, human corneal epithelial progenitors, and human dermal fibroblasts. These convenient sources of progenitor cell types have been fully characterized and have been found to retain their ability to differentiate. Each cell system is available as a starter cell culture with the appropriate CELLnTEC media kit for guaranteed results.

Description	Qty/Pk	Cat. No.
Human Epidermal Keratinocyte Progenitors (from pooled donors) & Media Kit	0.5 x 10^6 cells plus CnT-57 media	HPEKP.05
Human Epidermal Keratinocyte Progenitors (from pooled donors) & Media Kit	1.5 x 10^6 cells plus CnT-57 media	HPEKP.15
Human Epidermal Keratinocyte Progenitors (from single donor) & Media Kit	0.5 x 10^6 cells plus CnT-57 media	HPEKS.05
Human Epidermal Keratinocyte Progenitors (from single donor) & Media Kit	1.5 x 10^6 cells plus CnT-57 media	HPEKS.15
Human Corneal Epithelium Progenitors & Media Kit	0.5 x 10^6 cells plus CnT-20 media	HCEP-05
Human Corneal Epithelium Progenitors & Media Kit	0.5 x 10^6 cells plus CnT-58 media	HBEP-05
Human Dermal Fibroblasts & Media Kit	0.5 x 10^6 cells plus CnT-05 media	HDFS-05

Cardiac Stem Cell Isolation Kit

The Cardiac Stem Cell Isolation Kit uses Steriflip Nylon Net filters to efficiently separate dissociated cells from murine ventricular tissues. Differential gradient solutions eliminate the need for expensive cell sorting equipment by rapidly purifying Sca-1 positive CSCs. This method yielded an average 1.2 x 10^6 cells/mL with a Sca-1 purity of 69%, compared to only 11-14% with traditional methods of dissociation and filtration[2,3].

Description	Qty/Pk	Cat. No.
Cardiac Stem Cell Isolation Kit	5 isolations	SCR061
Cardiac Stem Cell Maintenance Medium	500 mL	SCM101
Cardiomyocyte Differentiation Medium	500 mL	SCM102

Quantitative Alkaline Phosphatase Kit

This kit allows the complete and easy quantification of alkaline phosphatase (ALP) present within an embryonic stem cell culture using a convenient 96 well colorimetric assay. Under alkaline conditions, ALP can catalyze the hydrolysis of p-NPP into phosphate and p-nitrophenol, a yellow colored by-product of the catalytic reaction, which is proportional to the amount of alkaline phosphatase present and can be quantified in a spectrophotometer. This kit includes sufficient reagents (buffers, stop/wash solutions, substrate, and standard) for 100 reactions.

Description	Qty/Pk	Cat. No.
Quantitative Alkaline Phosphatase ES Characterization Kit	100 assays	SCR066

Human Neurogenesis Characterization Kit

Millipore's Human Embryonic Stem Cell Neurogenesis Characterization Kit contains a complete panel of validated antibodies that allow researchers to identify and quantify the extent of differentiation to specific neuronal subtypes from a starting culture of human embryonic stem cells. Pluripotent markers, OCT-4, SSEA-4, and Sox-2 are provided in the kit to aid in the characterization of the starting human embryonic stem cell culture. To characterize the transition of human ES cells from pluripotent to multipotent state with the potentiality restricted to cells of the neural lineage, Nestin and Sox-2 is provided. βIII-tubulin antibody is provided to mark all neuronal cells while GAD67, ChAT, and TH antibodies are provided to specifically identify GABAergic, cholinergic, and dopaminergic neurons, respectively. All of the antibodies provided in the kit have been tested and optimized for use in immunocytochemistry on human embryonic stem cells and human-derived neural stem cells.

Description	Qty/Pk	Cat. No.
Human Embryonic Stem Cell Neurogenesis Characterization Kit	1 kit (containing 8 antibodies)	SCR065

References

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Human Cardiomyocyte Characterization Kit

The new Cardiomyocyte Characterization Kit contains a collection of validated antibodies for the immunocytochemical characterization of cardiomyocytes, along with control IgG antibodies. This is a convenient tool for monitoring the differentiation of stem cells into cardiomyocytes. Included in the kit are antibodies that are critical to the structure (actinin and desmin), contractile function (tropomyosin and troponin), and homeostatic control (ANP) of cardiac myocytes to a variety of signals that increase blood pressure. The kit may be used on human, mouse and rat cells.

Description	Qty/Pk	Cat. No.
Cardiomyocyte Characterization Kit	1 kit	SCR059

Human Embryonic Stem Cell Antigen-1, clone 051007-4A5

As part of our commitment to providing innovative products for the advancement of stem cell research, Millipore is proud to announce the addition of anti-HESCA-1 (Human Embryonic Stem Cell Antigen-1, MAB4407) to our growing stem cell portfolio. HESCA-1 targets a unique cell surface antigen that is expressed in five undifferentiated hES cell lines, and has not been found to react with differentiated cell types in teratomas or feeder cells, such as MEF, D-551, and Hs27. This monoclonal antibody has been validated for flow cytometry, ICC, IHC, and IF applications.

Description	Qty/Pk	Cat. No.
Anti-HESCA-1, clone 051007-4A5	100 µg	MAB4407

Millicell® Culture Plate Inserts and Filter Plates

Millicell products allow cells to access media from both their apical and basolateral sides while in culture. Cell growth, structure, and function more closely mimic what occurs *in vivo* when grown on membrane substrates rather than plastic supports. Millicell inserts for 6-, 12- and 24-well receiver plates make it possible to easily study both sides of the cell monolayer, and are available in both hanging and standing designs. Millicell plates are available in 24- and 96-well varieties to simplify scale up and automation. Millipore also offers a variety of membranes to accomodate your specific needs.

Description	Qty/Pk	Cat. No.
Millicell Standing Cell Culture, 6 well CM 0.4 µm	50	PICM03050
Millicell Standing Cell Culture, 24 well PCF 0.4 µm	50	PIHP01250
Millicell Hanging Cell Culture, 12 well PET 8 µm	48	PIEP15R48
Millicell 24 Cell Culture Plate, 5 µm	5	PSHT010R5
Millicell 96 Cell Culture Insert Assembly with 96 well feeder tray, 0.4 µm PCF	5	PSHT004S5

Nylon Net Steriflip Unit

The Steriflip filter unit is a disposable, sterile filtration system used in the preparation of tissue culture media, buffers, microbiological media, and other aqueous solutions. The new, streamlined design provides easier cell manipulation and optimal performance in a variety of applications. The vacuum-assisted, closed system option ensures sample sterility while enabling faster separation of large volumes of cellular material and improved recovery. The nylon mesh allows for separation of larger volumes of cellular material while ensuring sample sterility and vacuum filtration. This improves recovery and lessens time to collect isolated cells. Four mesh sizes allow for optimal performance in stem cell, cardiomyocyte, neurological, and skeletal tissue applications.

Description	Qty/Pk	Cat. No.
Steriflip 100 µm Nylon Net	25/pk	SCNY00100
Steriflip 60 µm Nylon Net	25/pk	SCNY00060
Steriflip 40 µm Nylon Net	25/pk	SCNY00040
Steriflip 20 µm Nylon Net	25/pk	SCNY00020

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