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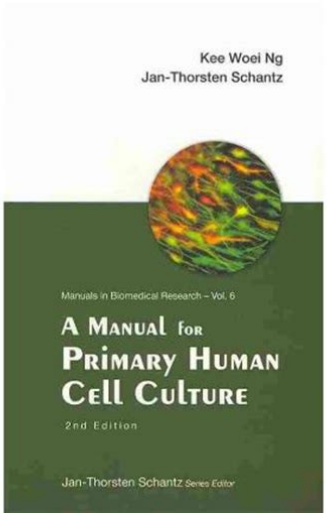

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By continuing to browse the site, you consent to the use of our cookies.In order to view the full content, please disable your ad blocker or whitelist our website www.worldscientific.com.During this period, our website will be offline for less than an hour but the Ecommerce and registration of new users may not be available for up to 4 hours.As in the first edition, the content of the manual is not exhaustive, but rather contains selected protocols for specific cell types from major tissue groupings in the body. This improved second edition also includes a new section on stem cells and additional material on transfection. It should serve as a foundation for individual researchers to experiment, explore, and establish niche protocols for their specific needs. With its compact physical format that makes it portable and flexible for usage in a laboratory setting, the manual will be a useful guide for all beginners in primary human cell culture work. The 13digit and 10digit formats both work. Please try again.Please try again.Well email you with an estimated delivery date as soon as we have more information. Your account will only be charged when we ship the item. Obtaining a viable culture from a tissue sample and maintaining it for experimental, diagnostic or therapeutic purposes can be quite a challenge. Based on laboratory protocols and practical experience from many years of primary cell culture, this manual presents the basic steps necessary for culturing primary human cells.Written by students for students, the manual serves well as a practical guide to primary human cell culture. The authors have left much space for notes and the design of the manual is such that it can be continuously upgraded and extended. The content of this manual is by no means exhaustive. Protocols for specific cell types, out of over 200 different cell types in the human body, were selected from major tissue groupings in the body.http://chakad-co.com/userfiles/canon-powershot-g9-manual.xml

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researchers.http://erimti.com/userfiles/canon-powershot-manual-focus.xml

Media formulation enhancements and cell maintenance protocols have also contributed to the explosion of primary cell use in research programs globally. The combination of these improvements has resulted in specialized research tools delivering straightforward and biorelevant data in a controlled, costeffective manner. This has enabled the scientific community to easily integrate primary cells into a diverse range of cellbased research projects. Why use primary cells instead of cell lines. How are primary cells currently being used in research. Who do I partner with and why. What additional services or solutions can cell providers offer. These are all important questions and the intended scope of this discussion. Leveraging the experience and expertise of a reputable primary cell provider delivers nothing but upside for clients, translating into significant savings in time and resources, and helping to shift focus back to developing worldclass therapies and research breakthroughs. Transformed cell lines Why change isn't always good. Transformed cell lines by definition have been altered or "transformed" in some manner, departing from the native cellular functions and growth characteristics found in normal primary cells. These altered cell lines typically exhibit abnormal or uncontrolled metabolic function and proliferative capacities in culture as a result of intracellular changes. Typically, cell lines are created or identified to fill particular research functions and have the benefits of lower initial cost, unlimited passages and elevated output signals for assay development. Cell lines can be specifically designed with a particular experimental function in mind by genetic manipulation to increase signal output or introduce targets into a cell normally devoid of them. In other cases, immortalized cell lines are collected as atypical cellular specimens from tumors and optimized for continued propagation as cell culture tools.

In many cases, immortalized cell lines simply bow to selective pressures in culture such as stress or overpassaging, that continually drive them further from a natural state without any methods to track or catalog changes as they occur. Unlike primary cell types, immortalized cell lines are considered to be nonterminally differentiated cells and never reach the final stage of cellular development that ultimately determines a tissue type. In addition, their unnatural state can result in altered growth characteristics, single or multiple genetic abnormalities, aberrant signaling pathways and a host of other detrimental characteristics. All these factors can generate misleading results and have catastrophic effects on subsequent research and development. Incorporating uncharacterized cell lines or cell lines from unknown sources or with unidentified genetic alterations can be a risky proposition. Primary cell types They're the real deal. Normal primary cells retain many if not all native cellular functions in vitro. Normal primary cells are terminally differentiated cells excluding stem or progenitor cells directly derived from a wellidentified tissue of origin. Primary cells are not genetically modified or altered in any way beyond natural exposure to environmental insults typically encountered during the lifespan of the organism from which the cells were isolated. Generally, primary cells retain normal morphology, cellular function, growth characteristics, cellular markers, signaling and genetic integrity when propagated in culture. However, isolating primary cells from multiple sources can introduce donortodonor variability in cellular characteristics, but for the most part; normal primary cells behave as expected and provide a controlled glimpse of native form and function crucial to understanding complex cell biology.

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Integrating primary cells into cellbased research programs has the potential to deliver biologically relevant and meaningful data because they maintain their fundamental cellular functions. Using primary cells has become more commonplace and in some cases has been mandated to receive data approval or further research funding. This is reflective of the push to incorporate more reliable cell culture tools into research and the emergence of high quality normal primary cells is at the forefront of these efforts. The vast majority of cell biologists would agree that normal primary cells are closest to the "real thing". Using a wellcharacterized, terminally differentiated cell type can deliver

confidence and assurance in data generated in vitro. These tools provide biorelevancy and more realistic experimental data over immortalized, transformed cell lines. Historically, cell lines have been useful research tools, however, they are subject to a number of inherent risks due to their altered or engineered nature creating an “artificial cellbased system”. More recently, the emergence of human and animal primary cells coupled with optimized media systems have opened research avenues not previously available. Researchers can now easily integrate wellidentified normal primary cell types from specific tissues into their projects. The finite lifespan of primary cells in culture is essentially a built in control mechanism protecting against the hazards of genetic alterations, transformations or a variety of selective pressures encountered by immortalized cell lines that are used for extended periods of time. In this effort to identify and control as many cell culture variables as possible, everything from the original tissue and cell types to cell culture media, reagents, supplements and other additives are being subjected to increased scrutiny.

Identifying their composition and performance characteristics helps develop legitimate, widely accepted in vitro research tools and systems to elucidate the complex functions, mechanisms, pathways and mysteries related to cell biology. Entrusting biomedical research to verified or more thoroughly characterized cell culture tools will deliver the most meaningful and trustworthy experimental results short of performing in vivo work. “Doityourself” characterization of cell lines or homemade primary cell cultures can be extremely costly, time consuming and can tie up valuable resources better used for actual exploratory research. Obtaining well qualified cells and media from a primary cell provider is an exceptional means to ensure fidelity and quality of results. Incorrectly identified or poorly verified cell culture tools call into question the integrity of the data they generate and can have a far reaching impact on biomedical research and development. Performing due diligence in identifying cell culture tools for use in research is vital to the future of research. This letter is one of the latest proposals within the scientific community to mitigate and eventually eliminate the detrimental practice of simply incorporating cell lines into research programs without first confirming the source, identity, and general function of cells being used. However, with cell line reliability called into question and newer options such as primary cells and media to conduct both fundamental and complex work, much of the unnecessary risk of culturing cell lines can be eliminated. Primary cells are “as advertised” by virtue of their production from known and wellidentified tissue types from human or animal origins. Of course, the variability associated with donortodonor differences exists with primary cells.

This can be advantageous; the donorspecific characteristics of primary cells can be an appropriate starting point for personalized medicine research and genomic analysis to pinpoint inherent differences contributing to a specific disease state. This is a valuable aspect of primary cells for drug development. However, it is also possible to reduce biological variability by pooling primary cells to average the respective donorderived responses. With alternatives to immortalized cell lines available in greater numbers and varieties, primary cells are a viable replacement to cell lines in many research programs. However, cost to use, maintenance in culture, and other unique challenges associated with primary cells should be considered. One must weigh the benefits of incurring some additional upfront costs to utilize primary cells vs.Primary cells can yield better data and reduce risk and overall cost by minimizing repeating experiments or validating cell line data in complementary primary cell types during subsequent research and development. Table 1 Pros and cons of primary cells vs.Several Yes. No. Retain functional enzymatic and signaling pathways of parent tissue. Potentially provide misleading data due to aberrant enzymatic or signaling pathway activities. Ideal for in vitro studies since it closely mimics in vivo activity. Potentially Challenging. Easily obtained in large amounts few limitations on application or verification. Easy. Can be complex and difficult,

especially in maintaining differentiated state. Not difficult to maintain or propagate cells. How are primary cells used. Where are they being used. Why With the advent of standardized, commercially available primary cell products, their use has increased in parallel with the research community's comfort level. To that end, basic as well as more complex signaling pathways lose their regulatory integrity and overall performance.

Incorporating native, terminally differentiated primary cell types essentially eliminates the loss of proper signaling function allowing a more thorough interrogation of cellular pathways. Without a standard by which to measure native cellular signaling pathways, meaningful data would be next to impossible to obtain. Both fresh, and more recently, cryopreserved hepatocytes are used for these applications. In addition, a large number of other normal primary cell types e.g. keratinocytes, microvascular endothelial cells have been used to investigate toxicity for drug development, environmental testing, or cosmetics and personal care evaluations. These unique tools have been used to investigate a number of indications including neural regeneration, bone marrow replenishment, soft tissue repair, burn therapies, and cardiac therapies. They also have the advantage of avoiding many of the ethical and legal issues associated with embryonic stem cells. Comparative studies vs. More specifically, measuring cellular activities and functions from cells derived from both normal and malignant tissues can unlock many mysteries of a disease such as cancer. Having the "normal" and "aberrant" in a sidebyside comparison is gaining traction and becoming one of the many ways to understand cellular transformations. This has allowed scalable cell provisioning at a reasonable cost per well to generate the cell numbers required to carry out both primary and secondary cellbased screening projects. Developing a primary cell gene expression "fingerprint" from different donors enables a more complete understanding of drug or compound effects and may help to identify specific donor variability. Primary cells are appropriate candidates for Labelfree cellular analysis since they are most reflective of the in vivo state. RNAi and miRNA studies using primary cells to assess cellular function and responses to treatment can be obtained on both a small and large scale using primary cells.

In depth interrogation of primary cells delivers a more complete and biorelevant representation of complex cellular processes that cannot be achieved using immortalized cell lines. In addition, the convergence of high content imaging methods and the use of complex assay readouts derives more detailed cellular information at a far more rapid rate maximizing the utility of a relatively limited resource. Commercial primary cell providers have developed elegant cell culture systems with optimized media and reagent formulations to address this segment of the life science research market. In addition, select commercial cell partners have the capabilities to provision primary cells at the large numbers necessary for these unique activities in a very costeffective method. Cost per well continues to drop enabling increased access to a wider variety of primary cells in larger numbers and at lower passages than before. Devoting cell culture expertise, materials and facilities to large scale primary cell provisioning by providers, delivers the right tools, in the right amounts without ramp up or additional infrastructure investment at a client site. Some of these cell providers are not only able to deliver cells and media for HCA and HTS applications, but can also provide related services to meet specific client requirements assay development, consultations, cell characterizations, donorspecific cell isolations, unique media formulations, and other customized deliverables. Cell companies used as outsourcing or "externalization" research partners can be very costeffective and allow clients to leverage resources internally and manage large, laborintensive cell culture projects very easily applying the appropriate cell culture focus and expertise. Partnering with a primary cell provider A match made at 37C. Commercial cell providers have made purchasing and integrating primary cells into biomedical research exceptionally easy.

In essence, cell companies have "demystified" primary cell culture whereas a few short years ago, primary cells were considered expensive and difficult to work with. Cell providers offer researchers

rapid, convenient and easytouse cell culture systems that can be used in virtually every cell biology application. Researchers now have a resource for information, instructions, protocols, methods and insight when tackling primary cell culture. Time and time again, clients cite their customer experience and the level of partnership and support they receive from their cell provider as the one differentiating factor that sets one company apart from another. Customer experience above even pricing is a major determining factor when partnering with a primary cell company. As with any complex research tool, there are a number of considerations after you have decided to incorporate primary cells into your project. One of the most vital decisions to make is where to acquire the cells. Choosing a provider for these unique systems can have a significant impact on the quality of your experience using primary cells and the value of the research data. Without question, obtaining and processing the raw materials for generating primary cells is time consuming, tedious and potentially quite expensive. Tremendous effort has been expended by commercial suppliers to identify and develop extensive tissue acquisition networks and contacts to procure the necessary biological materials tissues, organs, blood or other samples for producing top quality primary cells. To make sure that the proper tissue acquisition regulations and processing protocols are in place, the researcher should ask for documentation concerning donor consent along with IRB Institutional Review Board approval to ensure all ethical, legal and moral standards have been met in the process.

It is important to work with a primary cell partner that has dedicated the time and resources required to manage this key step in the process because it can greatly impact the quality of the product and their legal use. Your research depends on acquiring the right cells for the job. Because primary cells faithfully reflect the signaling and functionality of the donor tissue it is critical to have access to important donor parameters. A primary cell provider should be able to supply cells from a wide variety of donors allowing researchers to select the demographics age, gender, ethnicity, and disease state appropriate for their research. Additionally, since the cells have a finite lifespan in culture, it is very important to obtain cells at the lowest passage possible. By initiating experiments with low passage primary cells, the researcher can guard against potential performance problems due to extended time in culture and stress associated with in vitro experimentation. Cell providers should be able to supply passage number information with each of their products. Also, researchers should consider using specifically designed media formulations supplied by their cell provider, since they have been optimized for each cell system it’s the best assurance that the primary cell system will perform as intended. A wellformulated media system can minimize cellular stress, help to maintain the correct microenvironment and enable robust culture growth and survival of primary cells. Many cell systems come with a company pledge that the cell system will perform as indicated with full satisfaction guaranteed. It’s like buying insurance for your research. Quality control is a critical component of commercially available primary cell systems. Cells, media and reagents should all be subjected to a wide range of tests and qualification steps to ensure the proper function of each system.

Such vital checkpoints as the authentication of the cell type, passage number, growth capacity, doubling times, cell viability, cellspecific markers, and other mediarelated benchmarks should be assessed and available to the researcher to make an informed choice of providers. This QC is a wellcontrolled and exhaustive process that eliminates suboptimal cells, media and supplements, and adds a welcome level of assurance and peace of mind not usually found when researchers isolate primary cells inhouse. Working with a commercial cell provider that performs appropriate QC can prevent researchers from enduring high failure rates and paralyzing costs incurred from repeated inhouse isolation procedures to achieve the numbers of cells needed for experiments. Finally, with cell companies gaining critical expertise related to cellbased assays, many providers can offer clients additional services beyond cells and media to leverage resources and address unmet project demands. Researchers are expected to do more with less and the contraction trend in pharma and

biotech is leaving gaps in personnel and resources to keep pace with demands. These outsourcing opportunities enable clients of all sizes to “externalize” parts of or entire cellbased projects to wellqualified cell companies. Strategic outsourcing or consulting with these cell companies can minimize risk and save time and costs incurred by developing the necessary expertise internally. Partnering with the appropriate primary cell provider can quickly and efficiently advance cellbased programs by alleviating many pitfalls and delays associated with internal development. In many cases, technical teams from the cell provider can be an invaluable asset when planning, establishing and executing any size cellbased research project. Not all cell providers can or are willing to offer this level of support. Partnering with the right provider can make all the difference.

Conclusion The widespread availability of high quality primary cells and support reagents has opened up new avenues of cellbased and cell biology research. Improvements in cell isolation and cryopreservation, optimized media and reagents, and streamlined use protocols have allowed researchers to readily integrate robust primary cell systems into a wide variety of research programs. Commercial primary cell providers are bridging the gap to enable scientists to leverage their time and resources to focus on their own research and development. As primary cell providers continue to evolve their business models, they are increasingly able to deliver additional, valueadded services and support to primary cell clients large or small. The impact of primary cells on biomedical research cannot be denied. From basic cell biology to high throughput screening applications, primary cells are becoming a productive and integral component of many research programs. A partnership with right provider can synergize with your team to increase the pace and quality of your research. References 1. 2. 3. 4. 5. 6. 7. 8. 9. 10. CapesDavis, A., G. Theodosopoulos, I. Atkin, H.G. Drexler, A. Kohara, R.A. MacLeod, et al., Check your cultures. A list of crosscontaminated or misidentified cell lines. Int J Cancer, 2010. 1271 18. Hughes, P., D. Marshall, Y. Reid, H. Parkes, and C. Gelber, The costs of using unauthenticated, overpassaged cell lines how much more data do we need. Biotechniques, 2007. 435 575, 5778, 5812 passim. Cell line misidentification the beginning of the end. Nat Rev Cancer, 2010. 106 4418. Freshney, R.I., Database of misidentified cell lines. Int J Cancer, 2010. 1261 302. Romano, P., A. Manniello, O. Aresu, M. Armento, M. Cesaro, and B. Parodi, Cell Line Data Base structure and recent improvements towards molecular authentication of human cell lines. Nucleic Acids Res, 2009. 37Database issue D92532. Phuchareon, J., Y. Ohta, J.M. Woo, D.W. Eisele, and O.

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