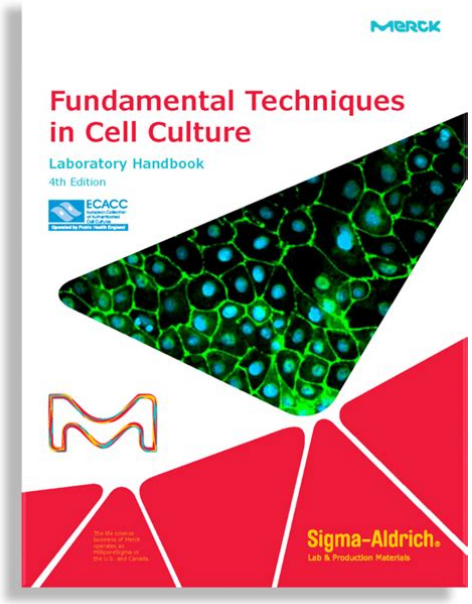

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By continuing to browse the site, you consent to the use of our cookies.In order to view the full content, please disable your ad blocker or whitelist our website www.worldscientific.com.During this period, our website will be offline for less than 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://luckylife68.com/images/upload/20200904164918_8bcb64494acd76fbbeb1ef6ef2d970ec.xml

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Hematopoietic cells derived from blood, spleen, or bone marrow as well as some transformed cell lines and cells derived from malignant tumors can be grown in suspension. Different cell types vary greatly with respect to their growth behavior and nutritional requirements. Optimization of cell culture conditions is necessary to ensure that cells are healthy and in optimal condition for downstream applications. Primary cultures are formed from cells that survive the disaggregation process, attach to the cell culture vessel or survive in suspension, and proliferate. These cultures are capable of only a limited number of cell divisions, after which they enter a nonproliferative state called senescence and eventually die out. Adherent primary cells are particularly susceptible to contact inhibition, that is, they will stop growing when they have reached confluency. At lower cell densities, however, the normal phenotype can be maintained. Primary cell culture is generally more difficult than culture of continuous cell lines. In addition, cell lines cultured for extended periods of time can undergo phenotypic and genotypic changes that can lead to discrepancies when comparing results from different laboratories using the same cell line. Furthermore, many cell types are not available as continuous cell lines. These cultures will proliferate for a limited number of cell divisions, after which they will senesce. The proliferative potential of some human finite cell cultures can be extended by introduction of viral transforming genes e.g., the SV40 transformingantigen genes. The phenotype of these cultures is intermediate between finite cultures and continuous cultures. The cells will proliferate for an extended time, but usually the culture will eventually cease dividing, similar to senescent primary cells. Use of such cells is sometimes easier than use of primary cell cultures, especially for generation of stably transfected clones.

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This alteration is commonly known as in vitro transformation or immortalization and frequently correlates with tumorigenicity. In contrast, human primary cell cultures rarely, if ever, become

immortal in this way and require additional genetic manipulation to form a continuous cell line. However, cell cultures derived from human tumors are often immortal. However, it should be remembered that these cells have undergone genetic alterations and their behavior in vitro may not represent the in vivo situation. All cell cultures are considered a biohazard because of their potential to harbor an infectious agent e.g., a virus. Primary cell cultures in particular should be handled carefully as these cultures have a high risk of containing undetected viruses. Although commonly used cell lines are generally assumed to be free of infectious agents, care should still be exercised when working with these cell lines as it is possible that they contain infectious agents, such as latent viruses. Cell cultures used to study specific viruses should be assumed to have the same degree of hazard as the virus under study. Work should be performed in an approved laminar flow hood using aseptic technique, and the creation of aerosols should be avoided see Handling cell cultures . After the work is complete, all waste media and equipment i.e., used flasks, pipets, etc. should be disinfected by autoclaving or immersion in a suitable disinfectant according to institutional and regional guidelines. Always use sterile equipment and reagents, and wash hands, reagent bottles, and work surfaces with a biocide or 70% ethanol before beginning work. To avoid aerosols, use TD to deliver pipets, and not TC to contain pipets; use pipets plugged with cotton; do not mix liquids by rapidly pipetting up and down; do not use excessive force to expel material from pipets; and do not bubble air through liquids with a pipet. Avoid releasing the contents of a pipet from a height into the receiving vessel.

Expel liquids as close as possible to the level of liquid of the receiving vessel, or allow the liquid to run down the sides of the vessel. For example, when using a centrifuge, ensure the vessel to be centrifuged is properly sealed, avoid drops of liquid near the top of the vessel, and use centrifuge buckets with caps and sealed centrifuge heads to prevent contamination by aerosols. Avoid placing laminar flow hoods near doorways, air vents, or locations where there is high activity. Hoods are often placed in dedicated cell culture rooms. Contamination of cell cultures can occur with both cell culture novices and experts. For example, cultures can be infected through poor handling, from contaminated media, reagents, and equipment e.g., pipets, and from microorganisms present in incubators, refrigerators, and laminar flow hoods, as well as on the skin of the worker and in cultures coming from other laboratories. To safeguard against accidental cell culture loss by contamination, we recommend freezing aliquots of cultured cells to reestablish the culture if necessary see Freezing and viability staining of cells . However, for some infections, no turbidity is observed and adverse effects on the cells are not easily observed. Mycoplasmal infections are one of the more common and difficulttodetect infections; their detection and eradication are described in further detail below. They are generally unaffected by the antibiotics commonly used against bacteria and fungi. Furthermore, as mycoplasma do not overgrow cell cultures and typically do not cause turbidity, they can go undetected for long periods of time and can easily spread to other cell cultures. The negative effects of mycoplasmal contamination include inhibition of metabolism and growth, as well as interference with nucleic acid synthesis and cell antigenicity.

Acute infection causes total deterioration of the cell culture, sometimes with a few apparently resistant colonies that may, in fact, also be chronically infected. There are two main approaches to detect mycoplasma — Hoechst 33258 staining 1, 3 and mycoplasmaspecific DNA probes. Alternatively, a PCRbased, mycoplasmatesting service is offered by the ATCC or other organizations on a feeforservice basis. Only if the cell culture is absolutely irreplaceable should eradication be attempted. This process should be performed by experienced personnel in an isolated hood that is not used for cell culture, preferably in a separate room. Elimination of mycoplasma is commonly achieved by treatment with various commercially available antibiotics such as a quinolone derivative

Mycoplasma Removal Agent, ciprofolxacin Ciprobay, enrofloxacin Baytril, and a combination of tiamulin and minocycline BMCyclin. Treatment procedures and appropriate antibiotic concentrations can be found in the suppliers' instructions and in references 1 and 3. To avoid crosscontamination, only use cell lines from a reputable cell bank; only work with one cell line at a time in the hood; use different pipets, bottles of reagents, and bottles of media for different cell lines; and check cells regularly for the correct morphological and growth characteristics. Different cell types have highly specific growth requirements, and the most suitable medium for each cell type must be determined experimentally. Common basal media include Eagle minimal essential medium MEM, Dulbecco's modified Eagle medium DMEM, RPMI 1640, and Ham F10. These contain a mixture of amino acids, glucose, salts, vitamins, and other nutrients, and are available either as a powder or as a liquid from various commercial suppliers. Serum is a partially undefined material that contains growth and attachment factors, and may show considerable variation in the ability to support growth of particular cells.

Fetal calf serum FCS is the most frequently used serum, but for some applications, less expensive sera such as horse or calf serum can be used. Different serum batches should be tested to find the best one for each cell type. Lglutamine is an unstable amino acid that, with time, converts to a form that cannot be used by cells, and should be added to medium just before use. Antibiotics and fungicides can be used as a supplement to aseptic technique to prevent microbial contamination. The working concentration of commonly used antibiotics and fungicides is provided in the tables Commonly used antibiotics for animal cell culture and Commonly used fungicides for animal cell culture. Some cell types, particularly primary cells, require additional supplements e.g., collagen and fibronectin, hormones such as estrogen, and growth factors such as epidermal growth factor and nerve growth factor to attach to the cell culture vessel and proliferate. If microbial growth has occurred after this incubation, the medium or supplement should be discarded. Cell cultures should be incubated in an incubator with a tightly regulated temperature e.g., a waterjacketed incubator and CO 2 concentration.Sterile, disposable dishes and flasks that have been treated to allow attachment of animal cells to the growing surface are available commercially. The working cell bank comprises cells from one of the master bank samples, which have been grown for several passages before storage. If future cell samples are needed, they are taken from the working cell bank. The master cell bank is used only when absolutely necessary. This ensures that a stock of cells with a low passage number is maintained, and avoids genetic variation within the cell culture. This instability can result from variations in cell culture conditions, genomic variation, and selective overgrowth of constituents of the cell population.

We recommend using cells with a low passage number Cultures should be examined regularly to check for signs of contamination and to determine if the culture needs feeding or passaging. These protocols are examples of methods for general cell culture, and have not been rigorously validated and optimized by QIAGEN. There are many alternative protocols in current use. IMPORTANT Wear protective goggles and gloves when thawing vials that have been stored in liquid nitrogen. Vials may explode when removed from liquid nitrogen. IMPORTANT Proceed to step 4 as soon as the cells have thawed. Do not allow the cells to warm up before transferring them into growth medium. Swirl the vessel gently to mix the cells with the medium. Note Immediate removal of DMSO may sometimes be necessary, especially for suspension cells, primary cells, and sensitive cell types. For such cell types, pipet the thawed cell suspension into a sterile centrifuge tube containing prewarmed medium, centrifuge at 200 x g for 2 min, aspirate the supernatant, resuspend the cells in fresh growth medium, and then transfer to an appropriate cell culture vessel. IMPORTANT Thoroughly mix the cells in the cell culture vessel to ensure even distribution of the cells throughout the vessel. This procedure is performed whenever the cells need to be harvested e.g., for passaging, counting, or for nucleic acid isolation. Do not force the cells to detach before they are ready to do so, or clumping may occur. Overly confluent cultures, senescent cells, and some cell lines may be difficult to

trypsinize. While increasing the time of trypsin exposure may help to dislodge resistant cells, some cell types are very sensitive to trypsin and extended exposure may result in cell death. In addition, some cell lines will resist this treatment and will produce cell clumps. Use medium containing the same percentage of serum as used for growing the cells. The serum inactivates trypsin activity.

If pipetted too vigorously, the cells will become damaged. Ensure that pipetting does not create foam. Store at room temperature. Store at room temperature. Adherent cell cultures therefore need to be routinely passaged, that is, once the cells are confluent, a fraction of the cells need to be transferred to a new cell culture vessel. Suspension cells will exhaust their culture medium very quickly once the cell density becomes too high, so these cultures similarly require regular passaging. We do not recommend passaging adherent cell cultures more than once every 48 h. Resuspend the cells in an appropriate volume of prewarmed growth medium containing serum. The volume of medium used to resuspend the cells depends on the split ratio required see step 2 and the size of the cell culture vessel. If too small a volume is used, it may be difficult to accurately pipet the desired volume to the new culture vessel. Conversely, if too large a volume is used, the culture vessel may be too full following transfer of the cells. Removal of trypsin may sometimes be necessary following harvesting of adherent cells, especially for primary and sensitive cell types. Centrifuge the cells at 200 x g for 5 min, carefully aspirate the supernatant, and resuspend the cells in an appropriate volume of prewarmed medium containing serum. Swirl the vessel gently to mix the cells with the medium. IMPORTANT Thoroughly mix the cells in the cell culture vessel to ensure even distribution of cells. IMPORTANT Some cell types will not survive if too few cells are transferred. We do not recommend high split ratios for primary cells, sensitive cell types, or senescent cultures. For adherent cells, we recommend adding enough cells so that the culture takes approximately one week to reach confluence again. This minimizes the number of times the cells are trypsinized as well as the handling time required to maintain the culture.

When determining how many cells to transfer to the new cell culture vessel, it can be helpful to think in terms of how many cell divisions will be required for the culture to reach confluence again. For example, if half the cells are transferred, then it will take the culture one cell division to reach confluency again; if a quarter of the cells are transferred then it will take 2 cell divisions, and so on. If a culture divides once every 30 h or so, then in one week it will undergo approximately 5 cell divisions. A split ratio of 132 125 should therefore be appropriate for the cells to reach confluency in about one week. In step 1, resuspend the cells in 8 ml medium, and transfer 0.25 ml to the new cell culture vessel. One method for counting cells is to use a hemocytometer. A hemocytometer contains 2 chambers see figure Counting cells using a hemocytometer . Cell concentration is determined by counting the number of cells within a defined area of known depth volume. Dry with lens paper. It is important that the coverslip is properly attached to obtain the correct chamber depth. The appearance of Newton’s rings bright and dark rings caused by interference in the air between the coverslip and the glass surface of the hemocytometer will confirm that the coverslip is attached properly. Resuspend the cells in an appropriate volume of prewarmed growth medium. Tip It may be necessary to centrifuge cells and resuspend in a smaller volume to obtain the desired cell concentration for counting. For adherent cells, it is important to produce a singlecell suspension after trypsinizing. Cell clumping will make counting difficult and inaccurate. Repeat for the second chamber. The cell distribution should be homogeneous in both chambers. The cell suspension is drawn under the coverslip and into the chamber by capillary action. The cell suspension should just fill the chamber. Blot off any surplus fluid without disturbing the sample underneath the coverslip.

Count the total number of cells in 5 of the 9 major squares. Count cells that overlap the top and left border of squares but not those overlapping bottom and right borders. This prevents counting overlapping cells twice. If the cell density is too high, the cell suspension should be diluted, noting the dilution factor. Dry with lens paper. The working cell bank comprises cells from one of the

master bank samples, which have been grown for several passages before storage. If and when future cell samples are needed, they are taken from the working cell bank. This ensures that a stock of cells with a low passage number is maintained, and avoids genetic variation within the culture. Adherent and suspension cell cultures should not be at a high density for freezing. We recommend freezing cells when they are in the logarithmic growth phase. IMPORTANT Freezing medium containing DMSO is hazardous and should be handled with caution. Label vials with the name of cell line, date, passage number, and growth medium. Tip It may also be useful to note the cell density in the freezing vials before storing. This enables determination of the cell density that provides optimal recovery after thawing. A controlledrate freezing device can be used instead of the polystyrene box and cotton wool method. This staining method is based on “dye exclusion” cells with intact membranes exclude i.e., do not take up the dye and are considered viable. Alternatively, add 0.4 ml trypan blue directly to 0.4 ml of cells in growth medium.Cold Spring Harbor, NY Cold Spring Harbor Laboratory Press. New York Wiley Interscience. How to make and transform competent cells, how to culture and handle plasmidcontaining cells, and commonly used techniques for analysis of genomic DNA.It also deals with RNAi and the use of siRNA, together with miRNA, mimics, and inhibitors.It also includes guidelines and suggestions for maximizing results from your PCR.

This is particularly useful for forensics and genetic disease research, where DNA quantities are limited, but many analyses are required. Various WGA techniques have been developed that differ both in their protocols, amplification accuracy, and easeofuse.These new sequencing platforms allow highthroughput sequencing for a wide range of applications. In addition to basic techniques, a wide range of specialised practical protocols covering the following areas are included cell proliferation and death, invitro models for cell differentiation, invitro models for toxicology and pharmacology, industrial application of animal cell culture, genetic manipulation and analysis of human and animal cells in culture. Only valid for books with an ebook version. Springer Reference Works and instructor copies are not included. By using our website you agree to our use of cookies. 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. 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. show more. Would you like to change to the United States site To download and read them, users must install the VitalSource Bookshelf Software. Ebooks have DRM protection on them, which means only the person who purchases and downloads the ebook can access it. Ebooks are nonreturnable and nonrefundable.This is a dummy description.This is a dummy description.This is a dummy description.This is a dummy description.This eagerly awaited edition reviews the increasing diversity of the applications of cell culture and the proliferation of specialized techniques, and provides an introduction to new subtopics in minireviews.

New features also include a new chapter on cell line authentication with a review of the major issues and appropriate protocols including DNA profiling and barcoding, as well as some new specialized protocols. Because of the continuing expansion of cell culture, and to keep the bulk of the book to a reasonable size, some specialized protocols are presented as supplementary material online. Culture of Animal Cells A Manual of Basic Technique and Specialized Applications, Seventh Edition provides the most accessible and comprehensive introduction available to the culture and experimental manipulation of animal cells. This text is an indispensable resource for those in or entering the field, including academic research scientists, clinical and biopharmaceutical researchers, undergraduate and graduate students, cell and molecular biology and genetics lab managers, trainees and technicians. He is the author or editor of numerous books and a worldrenowned expert on cell culture techniques. Preparation and Sterilization, 173 10. Primary Culture, 207 11. Subculture and Cell Lines, 235 13. Authentication and Validation, 259 14. Microbial Contamination, 289 15. Cryopreservation and Banking, 307 16. Cloning and Selection, 327 16. Cell Sorting, 347 18. Cell

Line Characterization, 359 19. Differentiation, 383 20. Senescence, Immortalization, and Transformation, 455 23. Cytotoxicity, 513 24. Culture of Specific Cell Types, 531 25. Training Programs, 591 28. Problem Solving 627 29. By continuing to use this site you agree to our use of cookies. To find out more, see ourYou will only need to do this once.Culture media, animal tissue culture facilities and several cell characterization tools have emerged in modern biotechnology, particularly in the area of human health. In this chapter basics of animal tissue culture are discussed with a brief glimpse of the historical background, types of cultures, their maintenance and characterization tools involved in this process.

It also includes animal tissue cultures facilities and biosafety guidelines while working on mammalian cells under in vitro conditions. One of the most challenging tasks in animal tissue culture laboratory is to prevent contamination; thus this chapter also involves steps that must be considered to prevent contamination. Despite the various developments in animal cell and tissue culture since the late 1800s, until the early 1950s progress in animal tissue culture was stalled due to the nonavailability of a suitable cell line. In the early 1950s, for the first time, successful growth of cells derived from the cervical cancer of Mrs Henrietta Lacks was demonstrated. Animal cell culture is a significant tool for biological research. The importance of cell culture technology in biological science was realized a long time ago. Earlier dedifferentiation based experiments of cells due to selective overgrowth of fibroblasts resulted in the enhancement of culture techniques. Animal cell culture involves isolation of cells from a tissue before establishing a culture in a suitable artificial environment. Initial isolation of the cells from the tissues can be achieved by disaggregation using enzymatic or mechanical methods. The source of the isolated cells is usually an in vivo environment, but sometimes cells are also derived from an existing cell line or cell strain. Animal cell culture offers suitable model systems for investigating the following factors Drug screening and development. Mutagenesis and carcinogenesis. Normal physiology and biochemistry of cells. Potential effects of drugs and toxic compounds on the cells. In addition, it also permits reliable and reproducible results, and is thus considered as a significant model system in cellular and molecular biology. Mammalian cell culture requires an optimal environment for growth. Environmental conditions are divided into nutritional requirements and physicochemical requirements.