DOWNLOAD PDF CELL BIOLOGY PROTOCOLS

Chapter 1 : Protocols by Subject: Cell Biology | HSLS

Protocols. Cell Biology Laboratory Manual (William H. Heidcamp, Gustavus Adolphus College) A large collection of protocols related to cell biology work from microscopy to cell culture, histochemistry, etc. Highly recommended.

Not affected, but high intensity allows for smaller aperture Closing the aperture increases the resolution and the Z-sectioning effect Not affected directly but through the control of noise Image noise Not directly affected, but high intensity allows for lower gain Not directly affected, but large aperture allows for lower gain Raising the gain introduces more noise Photobleaching Higher intensity produces higher photobleaching Not affected Not affected the effects of these components on the collected image. Common problems and microscope care and maintenance Problems in light microscopy usually arise from misalignment or dirt on the optical surfaces. Dark image and uneven illumination usually mean that the condenser is not correctly centred or the condenser diaphragm or field diaphragm are closed down too much or off-centre. The opposite can also occur with the image visible, but pale and undefined; this usually means that too much light is coming through and causing flare. The field or condenser diaphragms need to be closed down and the condenser adjusted. Dirt on the eyepieces or the objective will cause blurring and loss of definition. Dust on the lenses should be blown away with dry air and the lens cleaned with lens tissue moistened with distilled water. Stubborn grease can be removed with xylol; alcohols may dissolve the lens cement. Immersion oil should be wiped off at the end of every session of microscope use with lens tissue; never let the immersion oil dry to form a hardened film. Immersion oil should not be allowed to come into contact with any lenses except the oil immersion objective, but it is surprising how often a film of oil is found on low and medium power objectives. This should be cleaned off as for the oil immersion objectives. Care should be taken not to scratch the lens surfaces. Care should also be taken to ensure that the tubes of the microscope are closed either by an eyepiece or a dust plug. Similarly all positions on the revolving nosepiece should also be closed with a dust plug. The rackwork and other moving parts should be treated with great care; it is essential not to force anything if it is apparently jammed. Do not apply grease of an unspecified type to the sliding surfaces of the course-focusing adjustment or the gliding stage. At all times the instrument should handled carefully; for example, when carrying the microscope hold the base with one hand and the body with the other. When not in use the microscope should always be protected by a vinyl dust cover or kept in a cabinet. This can be done in a number of ways. Adherent cells can be grown on coverslips in Petri dishes or in special slide chambers. For easier handling suspension cells can be bound to slides using chemical linkers or by centrifuging onto slides using a cytocentrifuge [3]. This positively charged polymer binds to glass sides through the charged lysine side groups; cells which have an overall negative charge bind to the positively charged polymer through non-covalent interactions. Do not store for long periods; make fresh each week. Coat clean glass coverslips or slides by incubating in the poly-L-lysine solution for 10 min at room temperature. Wash in several changes of water and air-dry. Add a drop of cell suspension in PBS onto the coverslip. Incubate for 10 min Table 1. After this the cells are ready for fixing. Fixation The fixation process preserves the specimen and stabilizes the cellular structure while permeabilizing the cell to allow access of stains or antibodies, in the case of immunostaining [5, 6]. Fixatives can be divided into two broad categories, organic solvents and cross-linking reagents. The organic solvents such as acetone and alcohols extract lipids and dehydrate the cells; macromolecules are precipitated. Crosslinking agents, for example formaldehyde or glutaraldehyde, form cross-links mainly between amino groups, stabilizing molecular structures. The choice of fixative depends on the sample and staining techniques to be used; some common fixatives are listed in Table 1. The fixation process Some commonly used fixatives Suggested conditions 5 min, room temperature. Air-dry 5 min, room temperature. Air-dry 10 min, room temperature. Air-dry for immunocytochemistry remove solvent and wash several times with PBS 10 minutes, room temperature. Also the procedures and conditions should be carefully controlled for consistency. Preparation of tissue sections Two commonly used methods used to prepare tissue for staining are sectioning of paraformaldehyde-fixed, paraffinembedded tissues and sectioning of frozen tissues [6, 7]. Frozen sectioning is a relatively gentle way to prepare tissue samples with the advantage that the

tissue is unfixed. A disadvantage is that specialist sectioning equipment in the form of a cryostat is required. Most histological studies are carried out on paraformaldehyde-fixed, paraffin-embedded tissue samples. The fixing and embedding processes are quite harsh and may not be suitable for immunohistochemistry where particular care has to be taken with fixation. Staining Fixed cultured cells, cell smears or tissues sections need to be stained to give the contrast required for observation with bright field microscopy. There are many different stains that can be used to differentiate different cell types and different subcellular structures [3, 5, 6]. A selection of commonly used stains is given in Table 1. Most of these stains are commercially available, prepared as stable solutions. Bios Scientific Publishers, Oxford. Academic Press, New York. Theoretical Background and Practice. Robin Harris Protocol 2. When used appropriately, EM is able to provide direct visual evidence for the organization of biological structures at the subcellular and even molecular level. This chapter aims to provide cell biologists with some basic knowledge of the available EM specimen preparation techniques which will allow them to carry out the more straightforward analyses of cellular, subcellular and macromolecular samples. For more detailed methodologies the reader should consult one of the several available texts that is dedicated to EM [1â€”5]. The technique of freeze-fracture, although widely used in the past, is somewhat less popular today although it remains useful for a number of situations [1, 5]; it is not easy for the inexperienced to perform as a routine procedure and is not included in this chapter. Thin sectioning of resin embedded cell suspensions, monolayers and organelles see Protocol 2. Suspended cells and isolated organelles can be pelleted, followed by dispersal in low melting temperature agarose see Protocol 2. Agarose can also be added to monolayers in situ, for direct processing on the plastic cell culture flask, miniature culture system or Petri dish. This agarose encapsulation approach is considered to be especially convenient, since small pieces of gelled agarose, containing the cellular material of interest, can be processed throughout the specimen preparation stages far more easily than by repeated centrifugal pelleting and resuspension, prior to embedding of the fixed and dehydrated material in resin. A specialist technique for embedment-free electron microscopy is given in Protocol 2. Negative staining Negative staining see Protocols 2. When it comes to isolated organelles and their subfractions, such as mitochondria, chloroplasts, plasma membrane fractions such as cell junctions, rough and smooth ER, caveosomes, Golgi, nuclear envelope, cytoskeletal and fibrillar proteins, negative staining has considerable potential. The same applies to the use of negative staining for the study of oligomeric proteins, enzymes and macromolecular assemblies such as the 20S and 26S proteasome, ribosomes and the isolated nuclear pore complex [3]. A recently introduced improvement to the technique provides a standardized procedure for spreading biological particulates, supported by negative stain alone, across the holes of holey carbon support films [6]. Vitrification The technique of vitrification of unstained biological particles suspended in a thin aqueous film, followed by cryoelectron microscopy, generally provides a superior structural approach than negative staining see Protocol 2. Cryoelectron microscopy brings with it some technical difficulties, but these have largely been overcome in recent years [7]. This low temperature negative staining approach is somewhat more easily performed than the study of unstained vitrified specimens and can yield a resolution in the order of 1. Metal shadowing and freeze-fracture Both negatively stained and vitrified unstained specimen preparation is usually performed with unfixed samples. On the other hand, platinumâ€”carbon or tungstenâ€”iridium metal shadowing often requires prior fixation of the biological material, together with total removal of fixative and buffer salts by prior dialysis against distilled water, washing after attachment to a mica or carbon substrate, or suspension in a buffer composed of volatile salts such as ammonium acetate or ammonium bicarbonate usually together with glycerol see Protocol 2. The resolution obtained from metal shadowing used following freeze-fracture [5] is often somewhat inferior to negative staining and cryomicroscopy of unstained vitrified material, but excellent results have been achieved from freeze-dried and freeze-cleaved samples, where the granularity of the metal evaporated in vacuo is very fine [9]. Immunolabelling Of considerable significance in modern cell biological studies is the ability to perform immunolabelling of antigens located within cellular structures and isolated macromolecules. Colloidal gold particles ranging from 1 to 20 nm diameter are available commercially, which also enable double labelling procedures to be performed on the same tissue, using antibodyâ€”gold probes of different size. The preservation of antigenicity is a major consideration when post-embedding is to be performed. An underlying

difficulty of the post-embedding labelling procedures in the fact that the antigenic epitopes under investigation may not withstand the high concentrations of fixatives e. Consequently 2 percent para formaldehydeâ€”0. These probes can be chemically linked to antibodies and streptavidin and are likely to make an increasing impact within the areas of high-resolution cellular and macromolecular labelling in the future. Very often such staff may provide a service role, available to all users of the laboratory, dependent upon local collaborative arrangements and funding. Equipment and reagent hazards The EM preparative equipment available in different laboratories will vary somewhat with respect to the larger items, such as vacuum coating units, glow-discharge equipment, ultramicrotomes, cryoultramicrotomes, rapid freezing apparatus and cryostorage systems. The smaller cheaper items of equipment tend to be widely available in all laboratories, having been supplied through the international network of well-established suppliers of EM equipment and consumables. Some of the reagents used for electron microscopy are hazardous. In particular, osmic acid should be used with care and always within a fume extraction hood. All contaminated organic solvent waste should be disposed of in bulk via environmentally acceptable procedures. Uranyl acetate has a low level of natural radioactivity. Accordingly, waste solution and contaminated filter paper and tissues should be disposed of using specified, approved routes. Other waste heavy metal staining salts should be disposed of in accordance with local regulations. Immerse a clean dry microscope slide into the formvar solution. Allow slide to drain vertically onto a filter paper and then dry. Score three edges of one side of the slide with a single-sided razor blade and float off the formvar film onto a water surface i. Allow to dry and then carbon-coat to an 4 optimal thickness e. For production of support films of carbon alone, dissolve the formvar and wash away by immersing individual grids vertically into chloroform. Alternatively for carbon support films: Carbon-coat pieces of freshly cleaved mica with a layer of carbon see step 4, above. Float off the carbon onto the water surface, as step 3, above, position over the grids and lower the water level to bring the carbon onto the grids. Remove carefully and allow to dry beneath an 6 angle lamp before use. Alternatively, a calibrated quartz crystal, carbon thickness monitor may be available. This procedure will take approximately 1â€”2 h. Store in a well-stoppered bottle and avoid contamination with airborne dust. The former are, however, more often used for negative staining, but the latter may be found to be desirable for immunonegative staining using nickel or gold EM grids where an increased number of incubations and washing stages are necessary. Pause point 1 Carbon-coated mica can be stored under dust-free conditions until required.

DOWNLOAD PDF CELL BIOLOGY PROTOCOLS

Chapter 2 : Cell Biology/Cell Culture Protocols

Comprehensive lab protocols resource for the world of biology & life sciences research. BioLabProtocols includes animal techniques, biotechnology, biochemistry, cell biology, microbiology, molecular biology, immunology, plant biology, general lab techniques, troubleshooting tips, research methods and all other laboratory protocols.

Adam Bohnert , date: Despite these many tools, robust staining techniques for imaging germ cells Peipei Guo and Shahin Rafii , date: Generation of durable ex vivo vascular niche that maintains EC identity and preserves the angiocrine profile of organ of origin offers Gal Haimovich and Jeffrey E. Here, we describe a single-molecule fluorescent in situ hybridization smFISH method as performed in Haimovich et al. Therefore, there is a demand to perform live-cell imaging and to measure endogenous protein dynamics in single cells. However, fluorescent labeling of endogenous Neha Tandon , Kaushik N. They are used for both gene down-regulation by using shRNA or for gene up-regulation by using ORF of gene of interest. The technique of generating stable cell lines using 3rd generation lentivirus is very robust and it typically takes about However, cell line based models do not reflect the complexity of human tissues. We thus developed an inducible expression system for gene expression in ex vivo human tissues, which maintain native tissue architecture, such as epithelia and Linn , Sarah E. Webster and Mark K. Recently, however, studies have demonstrated that eye drops can reach the retina in the back of the eye if pharmacological agents are carried in appropriate vehicles. Here, we introduce an eye drop procedure to deliver a drug PNU , in combination Leblanc , Kevin M. Anderson , Mingqing Chen and Shuiying Hu , date: The purpose of this protocol is to describe a serial bleeding protocol, obtaining blood samples at six time points from single mouse Lee , Leah M. Weinstein , Carlos J. Samuda , Nicole A. Singer , Matthew D. Kutyna , Frank D. Kolodgie , Renu Virmani and Aloke V. Previous work, including ours, has focused on the role of intraplaque hemorrhage, particularly from immature microvessel angiogenesis, as an important contributor to plaque progression via increases in vascular permeability leading to further intraplaque hemorrhage,

Chapter 3 : Current Protocols in Cell Biology | Cell & Molecular Biology | Life Sciences | Subjects | Wiley

Cell Biology Laboratory Manual (Gustavus Adolphus College) is a comprehensive resource for basic cell biology methods, including cell fractionation, electrophoresis, microscopy, cell culture and differentiation experiments.

Chapter 4 : Current Protocols - Wikipedia

Forgot your password? Enter your email address below. If your address has been previously registered, you will receive an email with instructions on how to reset your password.

Chapter 5 : CSH Protocols -- Cell Biology

Protocols by Subject / Cell Biology. Cell Biology, general ; Analysis of Gene Expression in Cultured Cells Cell Culture; Cell Imaging; DNA Delivery/Gene Transfer.

Chapter 6 : Cell biology | Abcam

This book contains numerous usefulprotocols, covering light and electron microscopy, cell culture,cell separation, subcellular fractionation, organelle and membraneisolation, and the use of in vitro reassembly systems inCell Biology. Many of these protocols feature helpful notes andsafety information for practical application.

Chapter 7 : PubMed Journals will be shut down | NCBI Insights

DOWNLOAD PDF CELL BIOLOGY PROTOCOLS

Cell Biology. Antibody: Apoptosis: Appendix: Bioinformatics: C. elegance: Cell Biology: Cell Cycle.

Chapter 8 : Cell Biology Protocols

GIBCOÂ® Cell Culture Basics is an introduction to cell culture, covering topics such as laboratory set-up, safety and aseptic technique. You'll also find cell culture protocols for passaging, freezing, and thawing cultured cells.

Chapter 9 : Cell Biology Protocols - PDF Free Download

The comprehensive lab protocols resource for the world of biology & life sciences research. BioLabProtocols includes animal techniques, biotechnology, biochemistry, cell biology, microbiology, molecular biology, immunology, plant biology, general lab techniques, troubleshooting tips, research methods and all other laboratory protocols.