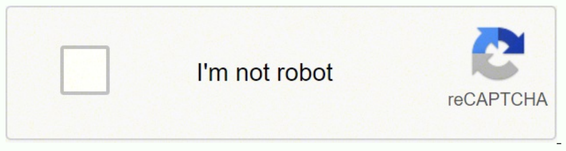

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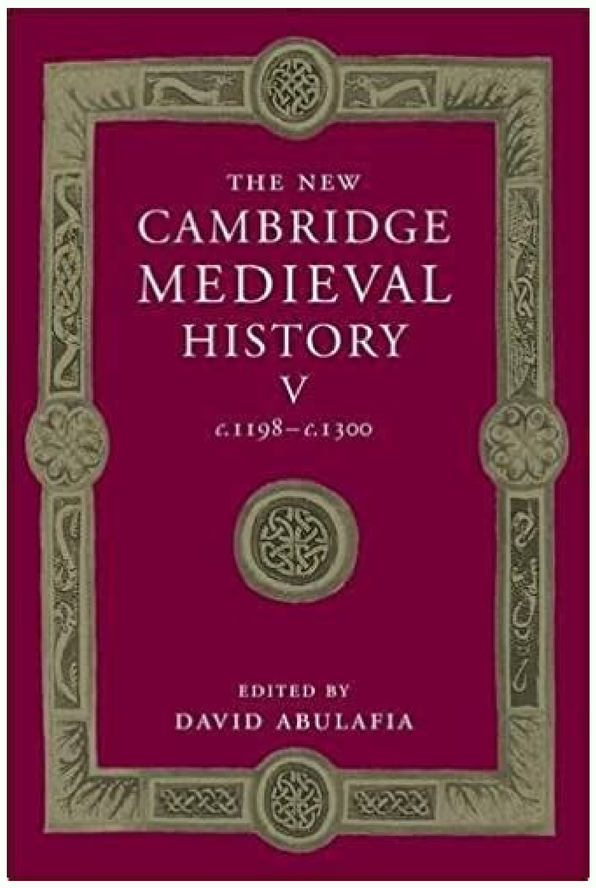

DMEM/F12 Medium is a 1:1 mixture of Dulbecco’s modified EMEM and Ham’s F-12. It is formulated with a reduced sodium bicarbonate concentration (1,500 mg/l) for use with 5% CO2 (see Sodium Bicarbonate and Buffering). As the cells metabolize and produce more CO2, the pH of the medium decreases as the chemical reaction below is driven to the right: H2O + CO2 ↔ H2CO3 ↔ H+ + HCO3- The optimal pH range of 7.2 to 7.4 can be maintained by supplementing the medium with sodium bicarbonate and regulating the level of CO2 in the atmosphere above the medium as shown by the reaction below: H2O + CO2 + NaHCO3 ↔ H+ + Na+ + 2HCO3- In tissue culture, cells are grown either in open systems (where there is free exchange of the atmosphere immediately above the medium with the atmosphere of the incubator) or in closed systems (where the two atmospheres are kept separate). Horse and bovine calf sera are less expensive and more readily available than fetal bovine serum. Heat inactivation is usually unnecessary and can be detrimental to the growth of some cells. Invertebrate cell culture media, such as Schneider’s Drosophila medium, may contain as much as 12.3 mM L-glutamine. For example, antibiotic use is recommended when developing and working with primary culture and when using flow cytometry to isolate subpopulations. Stock concentrations should be aliquoted into small volumes and stored at an appropriate temperature; most stock concentrations can be stored at –80°C, but check with your supplier prior to storing. BME was developed for culturing mouse L cells (ATCC CCL-1) and HeLa cells (ATCC CCL-2). For additional information regarding the preparation, storage, or usage of specific supplements, contact your local supplier or consult with the manufacturer’s Product Information Sheet. It can be very difficult to get these components to go back into solution after thawing, even if warmed to 37°C. ATCC offers the following three types of animal sera: These products are rigorously tested for adventitious infective agents and sourced from only U.S. herds. ATCC sera are routinely stored at −70°C. As with EMEM, there have been numerous modifications to the original formulation including Ham’s F-12 medium, a more complex formulation than the original F-10 suitable for serum-free propagation. Potassium nitrate was substituted for ferric nitrate. A few types of cell lines grow better in heat-inactivated sera such as embryonic stem cells16 and many insect cell lines.17 The following procedure can be used to heat-inactivate serum: Thaw serum. For serum-free media, reduce the concentrations by at least 50%. Heat inactivation of sera can also cause the formation of precipitates. Because EMEM is a simple medium, it is often fortified with additional supplements or higher levels of serum. The requirements for these components vary among cell lines, and these differences are partly responsible for the extensive number of medium formulations. HEPES buffer HEPES and other organic buffers can be used with many cell lines to effectively buffer the pH of the medium.8 Indeed, some standard medium formulations include HEPES. The temperature of the water bath will drop. Put the bottles in a 37°C water bath and gently agitate from time to time to mix the solutes that tend to concentrate at the bottom of the bottle. Do not keep the serum at 37°C any longer than necessary to thaw it, and do not thaw the serum at higher temperatures. Sodium pyruvate Pyruvate is an intermediary organic acid metabolite in glycolysis and the first component of the Embden-Meyerhof pathway. It will reduce or destroy growth factors present in the serum. After supplements have been added to a base medium, the shelf life of the complete growth medium should be determined on a case-by-case basis. Hybri-Care Medium (ATCC 46-X) is a combination and modification of DMEM and NCTC 135 medium supplemented with insulin, oxalacetic acid, and HEPES. When the temperature of the water bath reaches 56°C again, continue to heat for an additional 30 minutes. ATCC DMEM/F12 medium (ATCC 30-2006) has a reduced sodium bicarbonate concentration (1,500 mg/L) for use with 5% CO2. Media formulations Formulations of media available from ATCC can be found online. Otherwise the cells may be subject to metabolic stress which will impair their performance. These components include hormones, growth factors and signaling substances that sustain proliferation and maintain normal cell metabolism. In these cases, it must be aseptically added prior to use. In some cases, antibiotic use for short periods of time can serve as a valuable prophylactic. The exact composition is unknown and varies from lot to lot, although lot-to-lot consistency has improved in recent years. Sodium pyruvate is added to give a final concentration of 1 mM in most media, but is increased to 5 mM in Leibovitz’s L-15 medium primarily to facilitate use in CO2-free environments. Most commercially available liquid media report osmolality and it is advisable to check the osmolality of any medium after the addition of saline solutions, drugs or hormones dissolved in an acid or base solution, or large volumes of buffers (eg, HEPES). Over time, there have been numerous variations on the EMEM formula for different applications. Antibiotics can mask contamination by mycoplasma and resistant bacteria. Because L-glutamine is so labile, it is often omitted from commercial liquid medium preparations to lengthen the product shelf life. Carbohydrates are supplied primarily in the form of glucose. This precipitate may include crystals of calcium phosphate, but does not alter the performance of the serum as a supplement for cell culture. Do not freeze complete growth medium. Storage Store sera at −20°C or colder for storage over 30 days. Routine use of antibiotics or antimycotics for cell culture is not recommended unless they are specifically required, such as G418 for maintaining selective pressure on transfected cells. McCoy’s 5A and RPMI-1640 were developed at Roswell Park Memorial Institute (RPMI) in Buffalo, New York. Alternately, the concentration of L-glutamine can be measured directly by standard analytical means such as HPLC (High Performance Liquid Chromatography). Even if the contamination is eliminated, there is no way of ensuring that the resulting cell line will have the same characteristics as the initial one due to the stress of the treatment. L-Glutamine L-Glutamine (ATCC 30-2214) is an essential amino acid required by virtually all mammalian and insect cells grown in culture. It is based on the formulation used by David H. Antibiotics and antimycotics Antibiotics and/or antimycotic agents are added to cell culture media as a prophylactic to prevent contamination, as a cure once contamination is found, to induce the expression of recombinant proteins, or to maintain selective pressure on transfected cells. Open systems usually require the higher levels of sodium bicarbonate found in Earle’s salt solution combined with a 5 to 10% CO2 atmosphere supplied by the incubator. For most tissue culture work (pH 7.4), the medium should be bright red. Sera from fetal and calf bovine sources are commonly used to support the growth of cells in culture. Most complete growth media can be stored in aliquots at 2°C to 8°C for about a month. At low pH levels, phenol red turns the medium yellow, while at higher pH levels it turns the medium purple. Complete media containing protein supplements (eg, epidermal growth factor, bovine serum albumin, etc.) tend to degrade faster than base media alone. The culture vessel must be sealed (flasks tightly capped) to retain any CO2 generated by the cells. Osmolality The osmolality of cell culture media for most vertebrate cells is kept within a narrow range from 260 mOsm/kg to 320 mOsm/kg, even though most established cell lines will tolerate a rather large variation in osmotic pressure. Mycoplasma contamination in particular is very difficult to eliminate. ATCC RPMI-1640 (ATCC 30-2001) was modified to contain higher amounts of glucose (4,500 mg/L), sodium pyruvate, and HEPES buffer. Pyruvate may help in maintaining certain specialized cells, in clonal selection, in reducing the serum concentration of the medium,7 and in reducing fluorescent light-induced phototoxicity.10 Cellular metabolism of pyruvate produces carbon dioxide which is given off into the atmosphere and becomes bicarbonate in the medium. These media have the advantage of maintaining optimal pH in an open system when the culture vessel is removed from the enriched CO2 atmosphere of the incubator. In some cases, researchers “gas” the atmosphere of the culture vessel with a stream of sterile-filtered 5% CO2/95% air mixture and then tightly seal the flask prior to incubation in a nonhumidified and non-CO2 incubator.7 While these culture vessels work with simpler non-humidified, non-CO2 incubators, the medium requirements are those of an open system. It is used for protein production, as an energy source, and in nucleic acid metabolism. For most cell lines, ammonia toxicity is more critical for cell viability than L-glutamine limitation. For the few sensitive cell lines, use non-bovine sera or irradiated bovine sera. Unfortunately, phenol red can mimic the action of some steroid hormones, particularly estrogen. Other carbon sources include amino acids (particularly L-glutamine) and pyruvate. It can pass readily into or out of the cell. In closed systems the level of CO2 is regulated by the metabolism of the cells. Do not store sera at temperatures above −20°C for any length of time. Gentamicin sulfate, another antibiotic, is used at 50 to 100 µg/mL. RPMI-1640 will support the growth of a wide variety of cells in suspension as well as a number of cells grown as monolayers. L-Glutamine is not as labile in dry form and most powdered medium formulations do include it. Leibovitz’s L-15 Medium (ATCC 30-2008) is formulated for use without CO2 incubation as is found in teaching laboratories or when collecting biopsy samples. In addition to nutrients, the medium helps maintain the pH and osmolality in a culture system. Further, each lot is tested for its ability to support cell growth and is the same sera used in ATCC labs. In contrast to fetal or calf sera, horse serum is collected from a closed herd of adult animals ensuring lot-to-lot consistency. Avoid repeated freeze-thaws by dispensing and storing in aliquots. RPMI-1640 is a modification of McCoy’s 5A and was developed for the long-term culture of peripheral blood lymphocytes. The inclusion of the other non-essential amino acids (alanine, asparagine, aspartic acid, glycine, glutamic acid, proline, and serine) in some media formulations reduces the metabolic burden on the cells allowing for an increase in cellular proliferation. In open systems, humidity (to reduce evaporation) and a means of regulating CO2 levels (if the culture medium contains sodium bicarbonate) are required during incubation to maintain the pH of the culture medium. Bovine-derived products also may contain the agent responsible for bovine spongiform encephalopathy (BSE). The addition of supplements can change the final osmolality of the complete growth medium, which may have a negative effect on the growth of cells in culture. Heat inactivation ATCC does not routinely use heat-inactivated serum unless specifically required for a particular cell line. Sera will also buffer a complete medium. However, this compound can be toxic, especially for some differentiated cell types, so evaluate its effects before use.9 HEPES has been shown to greatly increase the sensitivity of media to the phototoxic effects induced by exposure to fluorescent light.10,11 Phenol red Phenol red is used to monitor the pH of media. All reputable suppliers test their products for infectious virus by several methods including fluorescent antibody, cytopathic effect, and hemadsorption. Use caution when adding more L-glutamine than is called for in the original medium formulation. Unfortunately, there is no test for the presence of this agent and we highly recommend that you obtain all bovine products (including sera) from countries not affected by BSE such as the United States, Australia, and New Zealand. L-Glutamine concentrations for mammalian cell culture media can vary from 0.68 mM in Medium 199 to 4 mM in Dulbecco’s Modified Eagle’s Medium. CO2 dissolves freely into the medium and reacts with water to form carbonic acid. While cell lines can be cured of microbial contamination with antibiotics and/or antimycotics, this is not recommend unless the cell line is irreplaceable; the process is lengthy and there is no guarantee contamination will be eliminated. ATCC DMEM (ATCC 30-2002) has 4,500 mg/L of glucose and a reduced sodium bicarbonate concentration (1,500 mg/L) for use with 5% CO2. Mix gently every 5 minutes to insure uniform heating. Horse serum is less likely to carry the contaminants found in bovine sera such as viruses and less likely to metabolize polyamines which may be mitogenic for some cells. Unfortunately, naturally derived products from bovine sources may contain adventitious viruses such as bovine viral diarrhea virus (BVDV), bovine parvovirus, bovine adenovirus, and blue tongue virus. Some medium formulations incorporate other buffering systems such as phosphate or HEPES in addition to CO2/sodium bicarbonate. Dulbecco’s Modified Eagle’s Medium (DMEM) has roughly twice the concentration of amino acids and four times the amount of vitamins as EMEM, as well as ferric nitrate, sodium pyruvate, and some supplementary amino acids (though not all nonessential amino acids). These products are also screened for the standard microbial contaminants such as bacteria, fungi, and mycoplasma. ATCC EMEM (ATCC 30-2003) contains Earle’s balanced salt solution, nonessential amino acids, and sodium pyruvate. Closed systems usually require media with buffers based on Hanks’ balanced salt solution having relatively low levels of sodium bicarbonate. Its addition to tissue culture medium provides both an energy source and a carbon skeleton for anabolic processes. If L-glutamine is suspected to be a limiting factor during cell culture, a simple test of ‘spiking’ the medium with a small amount of L-glutamine will determine whether or not more is required. Media supplements The complete growth media recommended for some cell lines requires the addition of components not already available in the base media and serum. ATCC IMDM (ATCC 30-2005) has a reduced sodium bicarbonate concentration (1,500 mg/L) for use with 5% CO2. At one time animal serum was a major source of mycoplasma contamination of tissue culture cells. While most commercial formulations of liquid media do contain the appropriate amount of sodium bicarbonate, it is generally omitted from the powdered form and needs to be added before use. Remove serum from water bath, cool quickly (slow cooling can sometimes reverse the inactivation of complement activity), and store at −20°C or colder. It is best to discard the cell line and start over with new stocks. In some cases, the addition of L-glutamine to complete cell culture medium can extend the usable life of the medium. It also contains a reduced concentration of sodium bicarbonate (1,500 mg/L) for use with 5% CO2. Most have a sodium bicarbonate concentration of 1.5 g/L and are supplemented with extra sodium pyruvate. Preheat a water bath to 56°C. Iscove’s Modified Dulbecco’s Medium (IMDM) was formulated for growth of lymphocytes and hybridomas. ATCC Ham’s F-12K (ATCC 30-2004) has a reduced sodium bicarbonate concentration (1,500 mg/L) for use with 5% CO2. Nonessential amino acids All medium formulations contain the ten essential amino acids as well as cysteine, glutamine, and tyrosine. Removal of complement is usually unnecessary, but can be important when preparing or assaying viruses or in cytotoxicity tests. Compared to DMEM, it has additional amino acids, vitamins and inorganic salts. Cell culture media are complex mixtures of salts, carbohydrates, vitamins, amino acids, metabolic precursors, growth factors, hormones, and trace elements. However, if any supplement is expected to expire before the one-month period has passed, the expiration date for the complete growth media should follow suit. In general, 1.2 g/L to 2.2 g/L of sodium bicarbonate is used with 5% CO2 whereas 3.7 g/L sodium bicarbonate is used with 10% CO2. ATCC recommends storing media between 2°C and 8°C, away from light. If the presence of flocculent material or turbidity is a concern, it can be removed by filtration through a 0.45-µm filter. Please note that there are cell lines in the collection that require media not currently sold by ATCC. L-Glutamine degradation results in the build-up of ammonia which can have a deleterious effect on some cell lines. For example, the snail embryo requires medium of about 155 mOsm/kg, while some insect cells prefer 360 mOsm/kg to 375 mOsm/kg. The original formulation contained 1,000 mg/L of glucose, but in the more commonly used variations this amount was increased to 4,500 mg/L. All ATCC media, with the exception of Leibovitz’s L-15 (ATCC 30-2008), are designed to be used with 5% CO2 levels. Turbidity and precipitates All sera may retain some fibrinogen. During cell growth, the medium changes color as it changes pH due to metabolites released by the cells. Some fastidious cell lines may require that components be added immediately before use. The presence of this material does not alter the serum’s performance. If an antibiotic is used in medium, penicillin-streptomycin solution (ATCC 30-2300) can be added at 0.5 to 1 mL of solution per 100 mL of cell culture medium for a final concentration of 50 to 100 IU/mL penicillin and 50 to 100 µg/mL streptomycin. In contrast, the osmolality requirements for some invertebrate cell lines fall outside of this range. The pH is maintained by one or more buffering systems; CO2/sodium bicarbonate, phosphate, and HEPES are the most common. Fetal serum is a rich source of growth factors and is appropriate for cell cloning and for the growth of fastidious cells. F-12K has increased amounts of amino acids, pyruvate, biotin, calcium, magnesium, putrescine, and phenol red in addition to other modifications from the F-12 formula. Consequently, closed systems provide additional protection against contamination and have simpler incubator requirements than open systems. It also contains HEPES and selenium. The buffering system employed in the medium needs to be matched to the culture system. The pricing and availability of fetal serum fluctuates considerably. It is an extremely rich and complex medium and will support the growth of a broad range of cell types in both serum and serum-free formulations. Phenol red, a pH indicator, is added to medium to colorimetrically monitor changes in pH. ATCC modification of McCoy’s 5A (ATCC 30-2007) has a slightly higher levels of sodium bicarbonate (2.2 g/L) and does not contain sodium pyruvate. Because external factors may initiate the conversion of fibrinogen to fibrin, flocculent material or turbidity may be observed after serum is thawed. Thawing serum in a bath above 40°C without mixing may lead to the formation of a precipitate inside the bottle. In some instances, glucose is replaced with galactose to decrease lactic acid build-up, as galactose is metabolized at a slower rate. The standard sodium bicarbonate/CO2 buffering system is replaced by a combination of phosphate buffers, free-base amino acids, higher levels of sodium pyruvate, and galactose. Cell cultures can be grown in CO2 incubators with L-15 medium provided there is no exchange between the air in the culture vessel with that of the incubator (ie, caps of flasks are tightly closed). Animal sera Sera serve as a source for amino acids, proteins, vitamins (particularly fat-soluble vitamins such as A, D, E, and K), carbohydrates, lipids, hormones, growth factors, minerals, and trace elements. Heat inactivation was originally performed to inactivate complement (a group of proteins present in sera that are part of the immune response) as well as to destroy mycoplasma contaminants. Simply add a small amount of L-glutamine (~2 mM final concentration) to the culture medium. For studies with estrogen-sensitive cells, such as mammary tissue, use media without phenol red. Ham’s Nutrient Mixtures were originally developed to support the clonal outgrowth of Chinese hamster ovary (CHO) cells (ATCC CCL-61). Fortunately, very few cell lines (except those of bovine origin) are susceptible to this virus. Thawing The following procedure is used to thaw serum: Place frozen serum in a refrigerator at 2°C to 8°C overnight. Today, mycoplasma contamination, if any, is removed by filtration. Some supplements may need to be dissolved in a solvent prior to subsequent dilution in serum-free medium to the stock concentration. Additionally, the sodium-potassium ion homeostasis is upset when phenol red is included in some serum-free formulations; this effect is neutralized by the inclusion of serum or bovine pituitary hormone in the medium.12 Phenol red is frequently omitted from studies with flow cytometry as its color interferes with detection. It is best to recheck the osmolality of the complete growth medium after small volumes of supplement stock solutions are added; optimal osmolality for most vertebrate cell lines should fall between 260 mOSM/kg and 320 mOSM/kg. Further, they can interfere with the metabolism of sensitive cells. The exact amount will depend upon the medium formulation. The rate and extent of L-glutamine degradation are related to storage temperatures, age of the product, and pH. The antimycotic amphotericin B is used at 2.5 µg/mL.13 These concentrations apply to media that contain serum. Kaighn’s modification of Ham’s F-12 (Ham’s F-12K) was designed to support the growth and differentiation of primary cells with or without serum. McCoy’s 5A (ATCC 30-2007) was originally used to grow Novikoff hepatoma cells and will support the growth of primary cultures. According to a study by HyClone,15 warming serum to 37°C inactivates heat-labile complement factors. Avoid antimycotics as they can be toxic to many cell lines. Supplements are usually prepared as 100× (or higher) stock solutions in serum-free medium. Media ingredients Sodium bicarbonate and buffering Cells produce and require small amounts of carbon dioxide for growth and survival.6 In culture media, dissolved CO2 is in equilibrium with bicarbonate ions and many medium formulations take advantage of this CO2/bicarbonate reaction to buffer the pH of the medium. Several ATCC cell lines were tested for BVDV contamination14 and the results of this study are indicated in the cell line description on the website. Freezing cell culture media at –70°C or below causes some of the growth factors and/or vitamins to precipitate out of solution. Sachs and collaborators5 for the propagation of hybridomas and other fastidious cell lines. However, nearly all sera today are filtered through several 0.1-µm pore (or smaller) filters which effectively remove this organism. BVDV, in contrast to the other virus contaminants, is present in nearly all bovine serum at very low levels even when tests for infectious virus are negative. Commonly used culture media include the following: Eagle’s Minimum Essential Medium (EMEM) was among the first widely used media and was formulated by Harry Eagle from his earlier and simpler basal medium (BME). Calf serum, because of its lower growth-promoting properties, is used in contact-inhibition studies with NIH/3T3 cells (ATCC CRL-1658). Use sufficient water to immerse the bottle above the level of serum. A precipitate can form in serum when incubated at 37°C or higher for prolonged periods of time which may be mistaken for microbial contamination. Mix thawed serum by gentle inversion and place in the 56°C bath. If the cell growth rate increases, L-glutamine is most likely deficient and more should be added. It is also more labile in liquid cell culture media than other amino acids. Additionally, serum buffers the culture medium, inactivates proteolytic enzymes, increases medium viscosity (which reduces shear stress during pipetting or stirring), and conditions the growth surface of the culture vessel.

14.05.2022 · The English version offers selected articles from the vernacular Asahi Shimbun, as well as extensive coverage of cool Japan,focusing on manga, travel and other timely news Download Full PDF Package. Translate PDF. Related Papers. INTRODUCTION: THEMES IN THE STUDY OF LIFE. By Ryan Lincay. Cell. By itsmiracle MoranWord. microtubules. By magendira mani vinayagam. Male and female gametes and fertilisation, ... Bioprocess Engineering Basic Concepts Second Edition. Malini Kanapathy. Download Download PDF. Full PDF Package Download Full PDF Package. This Paper. A short summary of this paper. 37 Full PDFs related to this paper. Read Paper. Download Download PDF. Sarcoidosis (also known as Besnier-Boeck-Schaumann disease) is a disease involving abnormal collections of inflammatory cells that form lumps known as granulomata. The disease usually begins in the lungs, skin, or lymph nodes. Less commonly affected are the eyes, liver, heart, and brain. Any organ, however, can be affected. The signs and symptoms depend on the organ ...

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