# Cell Culture Media

## Chemically defined medium

A **chemically defined medium** is a growth medium suitable for the in vitro cell culture of human or animal cells in which all of the chemical components are known. **Standard cell culture media** are commonly supplemented with animal serum (such as fetal bovine serum, FBS) as a source of nutrients and other ill-defined factors. The technical disadvantages to using serum include its undefined nature, batch-to-batch variability in composition, and the risk of contamination.

**There is a clear distinction between serum-free media and chemically defined media**. **Serum-free media** may contain undefined animal-derived products such as serum albumin (purified from blood), hydrolysates, growth factors, hormones, carrier proteins, and attachment factors. These undefined animal-derived products will contain complex contaminants, such as the lipid content of albumin. In contrast, **chemically defined media** require that all of the components must be identified and have their exact concentrations known. Therefore, a chemically defined medium must be entirely free of animal-derived components and cannot contain either fetal bovine serum, bovine serum albumin or human serum albumin.

### Classes of media

Animal culture media can be divided into six subsets based on the level of defined media (Jayme and Smith, 2000). From lowest definition to highest these are:

- Serum-containing media (commonly 10-20% FBS)
- Reduced-serum media (commonly 1-5% FBS)
- Serum-free media (synonymous with Defined media)
- Protein-free media (no protein but contains undefined peptides from plant hydrolysates)
- Chemically-defined media (with only recombinant proteins and/or hormones)
- Protein-free, chemically defined media (contains only low molecular weight constituents, but can contain synthetic peptides/hormones)
- Peptide-free, protein-free chemically defined media (contains only low molecular weight constituents)

### Components of cell culture media

| Component | Function |
|---|---|
| Carbon source (glucose/glutamine) | Source of energy |
| Amino acid | Building blocks of protein |
| Vitamins | Promote cell survival and growth |
| Balanced salt solution | An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc. |
| Phenol red dye | pH indicator. The color of phenol red changes from orange/red at pH 7-7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH. |
| Bicarbonate /HEPES buffer | It is used to maintain a balanced pH in the media |

**Typical Growth conditions**

| Parameter | |
|---|---|
| Temperature | 37 °C |
| CO2 | 5% |
| Relative Humidity | 95% |

## PHYSICOCHEMICAL PROPERTIES

### pH

Most cell lines grow well at pH 7.4. Phenol red is commonly used as an indicator. It is red at pH 7.4 and becomes orange at pH 7.0, yellow at pH 6.5, lemon yellow below pH 6.5, more pink at pH 7.6, and purple at pH 7.8.

### CO2 and Bicarbonate

Carbon dioxide in the gas phase dissolves in the medium, establishes equilibrium with $HCO_3^-$ ions, and lowers the pH. Because dissolved $CO_2$, $HCO_3^-$, and pH are all interrelated, it is difficult to determine the major direct effect of $CO_2$. The atmospheric $CO_2$ tension will regulate the concentration of dissolved $CO_2$ directly, as a function of temperature. This regulation in turn produces $H_2CO_3$, which dissociates according to the reaction:

$$H_2O + CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \qquad (1)$$

$HCO_3^-$ has a fairly low dissociation constant with most of the available cations so it tends to reassociate, leaving the medium acid. The net result of increasing atmospheric $CO_2$ is to depress the pH, so the effect of elevated $CO_2$ tension is neutralized by increasing the bicarbonate concentration:

$$NaHCO_3 \Leftrightarrow Na^+ + HCO_3^- \qquad (2)$$

The increased $HCO_3^-$ concentration pushes equation (1) to the left until equilibrium is reached at pH 7.4.

### Buffering

Culture media must be buffered under two sets of conditions: (1) open dishes, wherein the evolution of $CO_2$ causes the pH to rise, and (2) overproduction of $CO_2$ and lactic acid in transformed cell lines at high cell concentrations, when the pH will fall. A buffer may be incorporated into the medium to stabilize the pH. **HEPES is a much stronger buffer in the pH 7.2–7.6 range and is used at 10–20 mM**. It has been found that, when HEPES is used with exogenous $CO_2$, the HEPES concentration must be more than double that of the bicarbonate for adequate buffering.

### Oxygen

The other major significant constituent of the gas phase is oxygen. Whereas most cells require oxygen for respiration *in vivo*, cultured cells often rely mainly on glycolysis, a high proportion of which, as in transformed cells, may be anaerobic. Cells rely chiefly on dissolved $O_2$, which can be toxic due to the

elevation in the level of free radicals. Providing the correct $O_2$ tension is, therefore, always a compromise between fulfilling the respiratory requirement and avoiding toxicity. Strategies involving both elevated and reduced $O_2$ levels have been employed, as has the incorporation of free radical scavengers, such as glutathione, 2-mercaptoethanol ($\beta$-mercaptoethanol) or dithiothreitol, into the medium.

**Osmolality**

Most cultured cells have a fairly wide tolerance for osmotic pressure [Waymouth, 1970]. As the osmolality of human plasma is about 290 mosmol/kg, it is reasonable to assume that this level is the optimum for human cells *in vitro*, although it may be different for other species (e.g., around 310 mosmol/kg for mice [Waymouth, 1970]). In practice, osmolalities between 260 mosmol/kg and 320 mosmol/kg are quite acceptable for most cells but, once selected, should be kept consistent at ±10 mosmol/kg. Slightly hypotonic medium may be better for Petri dish or open-plate culture to compensate for evaporation during incubation.

**Temperature**

The optimal temperature for cell culture is dependent on (1) the body temperature of the animal from which the cells were obtained, (2) any anatomic variation in temperature (e.g., the temperature of the skin and testis may be lower than that of the rest of the body), and (3) the incorporation of a safety factor to allow for minor errors in regulating the incubator. Thus the temperature recommended for most human and warm-blooded animal cell lines is 37◦C, close to body heat, but set a little lower for safety, as overheating is a more serious problem than underheating.

Apart from its direct effect on cell growth, **the temperature will also influence pH due to the increased solubility of $CO_2$ at lower temperatures and, possibly, because of changes in ionization and the p*K*$_a$ of the buffer. The pH should be adjusted to 0.2 units lower at room temperature than at 37ºC**.

Other properties are: **Viscosity, Surface Tension and Foaming.**

## COMPLETE MEDIA

The term **complete medium** implies a medium that has had all its constituents and supplements added and is sufficient for the use specified. It is usually made up of a defined medium component, some of the constituents of which, such as glutamine, may be added just before use, and various supplements, such as serum, growth factors, or hormones.

**1. Amino Acids**

The essential amino acids are required by cultured cells, plus cystine and/or cysteine, arginine, glutamine, and tyrosine, although individual requirements for amino acids will vary from one cell type to another. Other nonessential amino acids are often added as well, to compensate either for a particular cell type's incapacity to make them or because they are made, but lost by leakage into the medium. Glutamine is required by most cells, although some cell lines will utilize glutamate; evidence suggests that glutamine is also used by cultured cells as a source of energy and carbon [Butler & Christie, 1994].

**2. Vitamins**

Water-soluble vitamins (the B group, plus choline, folic acid, inositol, and nicotinamide, but excluding biotin) are presumably derived from the serum. Biotin is present in most of the more complex media and p-aminobenzoic acid (PABA) is present in M199, CMRL 1066 (which was derived from M199), and RPMI

1640. All the fat-soluble vitamins (A, D, E, and K) are present only in M199, whereas vitamin A is present in LHC-9 and vitamin E in MCDB 110.

**3. Salts**

The salts are chiefly those of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Cl^-$,$SO_4^{2-}$, $PO_4^{3-}$, and $HCO_3^-$ and are the major components contributing to the osmolality of the medium. **Divalent cations, particularly $Ca^{2+}$, are required by some cell adhesion molecules, such as the cadherins. $Ca_2^+$ also acts as an intermediary in signal transduction and the concentration of $Ca^{2+}$ in the medium can influence whether cells will proliferate or differentiate.** $Na^+$, $K^+$, and $Cl^-$ regulate membrane potential, whereas $SO_4^{2-}$, $PO_4^{3-}$, and $HCO_3^-$ have roles as anions required by the matrix and nutritional precursors for macromolecules, as well as regulators of intracellular charge. **Calcium is reduced in suspension cultures in order to minimize cell aggregation and attachment.** The sodium bicarbonate concentration is determined by the concentration of CO2 in the gas phase and has a significant nutritional role in addition to its buffering capability.

**4. Glucose**

Glucose is included in most media as a source of energy. It is metabolized principally by glycolysis to form pyruvate, which may be converted to lactate or acetoacetate and may enter the citric acid cycle and is oxidized to form CO2 and water.

**5. Organic Supplements**

A variety of other compounds, including proteins, peptides, nucleosides, citric acid cycle intermediates, pyruvate, and lipids, appear in complex media.

**6. Hormones and Growth Factors**

Hormones and growth factors are not specified in the formulas of most regular media, although they are frequently added to serum-free media.

**7. Antibiotics**

Antibiotics were originally introduced into culture media to reduce the frequency of contamination. Though they have a number of significant disadvantages:

(1) They encourage the development of antibiotic resistant organisms.
(2) They hide the presence of low-level, cryptic contaminants that can become fully operative if the antibiotics are removed, the culture conditions change, or resistant strains develop.
(3) They may hide mycoplasma infections.
(4) They have antimetabolic effects that can cross-react with mammalian cells.
(5) They encourage poor aseptic technique.

A number of antibiotics used in tissue culture are moderately effective in controlling bacterial infections:

Amphotericin B, Ampicillin, Ciprofloxacin, Erythromycin, Gentamycin Gentamicin, Kanamycin, Neomycin, Nystatin, Penicillin-G, Polymixin B, Streptomycin $SO_4$, Tetracyclin, Tylosin

## SERUM

Serum contains growth factors, which promote cell proliferation, and adhesion factors and antitrypsin activity, which promote cell attachment. Serum is also a source of minerals, lipids, and hormones, many of which may be bound to protein. The sera used most in tissue culture are bovine calf, fetal bovine, adult horse, and human serum. Calf (CS) and fetal bovine (FBS) serum are the most widely used, the latter particularly for more demanding cell lines and for cloning.

**1. Protein**

Although proteins are a major component of serum, the functions of many proteins *in vitro* remain obscure; it may be that relatively few proteins are required other than as carriers for minerals, fatty acids, and hormones. Those proteins for which requirements have been found are **albumin**, which may be important as a carrier of lipids, minerals, and globulins; **fibronectin** (cold-insoluble globulin), which promotes cell attachment; and **α2-macroglobulin**, which inhibits trypsin. **Fetuin** in fetal serum enhances cell attachment and **transferrin** binds iron, making it less toxic and bioavailable. Other proteins, as yet uncharacterized, may be essential for cell attachment and growth. **Protein also increases the viscosity of the medium, reducing shear stress during pipetting and stirring, and may add to the medium's buffering capacity**.

**2. Growth Factors**

Platelet-derived growth factor (PDGF) is one of a family of polypeptides with mitogenic activity and is probably the major growth factor in serum. TGF-*β*, may inhibit growth or promote differentiation in epithelial cells. Other growth factors are fibroblast growth factors (FGFs), epidermal growth factor (EGF), endothelial cell growth factors such as vascular endothelial growth factor (VEGF) and angiogenin, and insulin-like growth factors IGF-I and IGF-II.

**3. Hormones**

Insulin promotes the uptake of glucose and amino acids and may owe its mitogenic effect to this property or to activity via the IGF-I receptor. Growth hormone may be present in serum—particularly fetal serum—and, in conjunction with the somatomedins (IGFs), may have a mitogenic effect. Hydrocortisone is also present in serum—particularly fetal bovine serum—in varying amounts and it can promote cell attachment and cell proliferation, but under certain conditions (e.g., at high cell density) may be cytostatic and can induce cell differentiation.

**4. Nutrients and Metabolites**

Serum may also contain amino acids, glucose, oxo (keto) acids, nucleosides, and a number of other nutrients and intermediarymetabolites.

**5. Lipids**

Linoleic acid, oleic acid, ethanolamine, and phosphoethanolamine are present in serum in small amounts, usually bound to proteins such as albumin.

**6. Minerals**

Serum replacement experiments [Ham & McKeehan, 1978] have also suggested that trace elements and iron, copper, and zinc may be bound to serum protein. Selenium probably helps to detoxify free radicals as a cofactor for GSH synthetase.

**7. Inhibitors**

Serum may contain substances that inhibit cell proliferation. Some of these may be artifacts of preparation (e.g., bacterial toxins from contamination before filtration, or antibodies, contained in the $\gamma$ -globulin fraction, that crossreact with surface epitopes on the cultured cells), but others may be physiological negative growth regulators, such as TGF-*β*.

# BALANCED SALT SOLUTIONS

A **balanced salt solution** (BSS) is a solution made to a physiological pH and isotonic salt concentration. It is composed of inorganic (sodium, potassium, calcium, magnesium, and chloride) salts and may include sodium bicarbonate and, in some cases, glucose. HEPES buffer (5–20 mM) may be added to these solutions if necessary and the equivalent amount of NaCl omitted to maintain the correct osmolality.

Balanced salt solutions are used for washing tissues and cells and are usually combined with other agents to treat the tissues and cells. They provide the cells with water and inorganic ions, while maintaining a physiological pH and osmotic pressure. Sometimes glucose is added as an energy source and phenol red is used as a pH indicator.

Eg: Hanks's BSS; Earle's BSS; Dulbecco's PBS [Without $Ca^{2+}$ and $Mg^{2+}$ (D-PBSA); With $Ca^{2+}$ and $Mg^{2+}$]