Chapter 1

Culture Conditions and Types of Growth Media for Mammalian Cells

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Basic requirement for culture medium

1.1.1. Nutritional components

Cells need the basic nutritional conditions to grow *in vitro*, including:

1. Amino acid

Amino acid is the raw material for the cell to synthesize protein. All the cells need twelve essential amino-acids: arginine, cystine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, tyrosine and valine, which are L-amino acids. Furthermore, glutamine is another component playing important role in the cell metabolism process. The nitrogen contained in glutamine is not only the source of purine and pyrimidine of nucleic acid, but also the essential material for the synthesis of the Tri-, bi-, mono-phosphate acid glycosides.

2. Monosaccharide

Cultured cells use aerobic glycolysis and anaerobic glycolysis of hexose as main energy source. In addition, hexose is used for the synthesis of some amino acid, fat and nucleic acid. Cell absorptive capacity varies among different monosaccharides, with the highest for glucose and the lowest for galactose.

3. Vitamin

Vitamins mainly act as coenzymes or prothetic groups in cell metabolism processes. Biotin, folate, nicotinamide, pantothenic acid, pyridoxine, riboflavin, thiamine and vitamin B12 are common component in culture medium.

4. Inorganic ion and trace element

Besides some basic elements (including sodium, potassium, calcium, magnesium, nitrogen and phosphorus), cell growth needs some trace elements, such as molybdenum, vanadium, iron, zinc and selenium, copper, manganese.

1.2. Somatomedin and hormones

Cells grown *in vivo* are always regulated by somatomedin and hormones. Many researches demonstrate that various somatomedin and hormones are very important to maintain cell function and status (differentiated or undifferentiated). Some hormones have promoting growth effects on different cell typs. For instance, insulin can promote the use of glucose and amino acids in the cell. Some hormones are cell-type specific, as hydrocortisone that can promote the growth of epidermal cells and prolaction that induces the proliferation of mammary epithelial cell.

1.3. Osmotic pressure

Cells need an isotonic environment and human plasma osmotic pressure is about 290 mOsm/kg, which is thought to be ideal osmotic pressure to culture human cells. Mouse plasma osmotic pressure is about 320 mOsm/kg. Osmotic pressure of 260-320 mOsm/kg fits for most mammalian cells.

1.4. pH

The suitable pH for most cells is 7.2-7.4; otherwise it will produce harmful effects. The culture medium should have some buffer capacity. The main substance causing pH changes is CO_2 produced in cell metabolism process. In an airtight environment, CO_2 can combine H_2O_2 to produce carbonic acid and thus reduce the pH value of the medium. Synthesized medium employs $NaHCO_3$-CO_2 buffer system to solve this problem. In the buffer system, the boost in [H+] increases the reaction rate H^+ + salt => weak acid and takes some H+ out of circulation. It is based on the constant equilibrium.

$$NaHCO3 + H_2O \longleftrightarrow Na^+ + HO^- + H_2O + CO_2 \uparrow$$

2. Natural medium

Natural medium is described as animal body fluids or medium of tissue extraction, including plasma, serum, lymph, chicken embryos leaching solution. Natural medium contains rich nutrients, various somatomedin and hormones, similar osmotic pressure and pH to body environment. As this medium has a very complicated production process and big batch-to-batch variation, the medium is gradually replaced by the synthetic medium. Today, serum is still the widely used natural medium.

2.1. Serum

2.1.1. Types of serum

Serum can derive from different animals. Current serum used in tissue culture is cattle serum. Human serum, horse serum is used for some specific cells. Cattle serum has several advantages as used in cell culture: adequate resource, mature preparation technique, long application time.

Cattle serum includes bovine calf serum, newborn calf serum and fetal bovine serum. Take sample for cattle serum from Gibco Life Technologies Company, fetal bovine serum derives from caesarean section fetal bovine; newborn calf serum comes from newborn calf born within 24h; bovine calf serum comes from calf with 10 to 30 days. Fetal bovine serum has highest quality because the fetal bovine doesn't expose to outside environment and has lowest antibodies and complement.

2.1.2. Main components of serum and its function

Serum is made from plasma by removing hemaleucin and contains various plasma protein, polypeptide, fat, carbohydrate, growth factor, hormones and inorganic mineral, etc. All these substances keep the physiological balance of promoting or inhibiting cell growth. The following table shows the main components of serum and their mean concentration.

Component	Mean concentration	Component	Mean concentration
Na^+	137mol/L	Alkaline phosphomonoesterase	225U/L
K^+	11 mol/L	Lactic dehydrogenase	860U/L
Cl^-	103 mol/L	Insulin	0.4μg/L
SeO_3^{2-}	26μg/L	Thyroid stimulator	1.2μg/L
Ca^{2+}	136mg/L	Folliclestimulating hormone	9.5μg/L
Fibonectin	35 mg/L	Bovine somatotropin	39μg/L
Urea acid	29 mg/L	Prolactin	17μg/L
Creatine	31mg/L	T_3	1.2μg/L
Hemoglobin	113 mg/L	Cholesterol	310μg/L
Bilirubin(total)	4 mg/L	Cortisone	0.5μg/L
Inorganic phosphorus	100mg/L	Testosterone	0.4μg/L
Glucose	1250mg/L	Progesterone	80μg/L
Urea	160mg/L	Prostaglandin E	6μg/L
Total protein	38g/L	Prostaglandin F	12μg/L
Albumin	23g/L	Vitamin A	90μg/L
α_2- macroglobulin	3g/L	Vitamin E	1 mg/L
Endotoxin	0.35μg/L	Fe^{2+},Zn^{2+}, Cu^{2+},Mn^{2+},Co^{2+}, Co^{3-},etc	μg/L to ng/L

Table 1. The main components of serum and their mean concentration.

The main function of serum is listed as follow:

a. Provide essential nutrients

Serum contains various amino acids, vitamins, inorganic minerals, fat, and nucleic acid derivatives, which are essential nutrients for cell growth.

b. Provide adherence and extension factor

Many cells cultured *in vitro* have to attach the culture vessel to grow, which is dependent on extracellular matrix. Cells can secrete extracellular matrix *in vivo*, but this ability will decrease or even disappear according to the increment of passages. Serum contains some components, (fibronection, laminin, etc), which can promote cell adherence.

c. Provide hormone and various growth factors

Serum contains various hormones, such as insulin, adrenocortical hormone (hydrocortisone, dexamethasone), steroid hormone (estradiol, testosterone, and progesterone), etc. The growth factors include fibroblast growth factor (FGF), epidermal growth factor (EGF), pleteletdericed growth factor (PDGF), and others.

d. Provide binding protein(s)

The binding proteins carry low molecular weight material. For example, the albumin carries vitamins, fat (fatty acid, cholesterol) and hormones. Transferrin carries iron.

e. Provide protection for some specific cells

Some cells (such as epithelial cells, myeloid cells) can release protease, which can be neutralized by the anti-protease ingredient in the serum. Serum is widely used to terminate the effect of the trypsin. Serum albumin facilitates the serum viscosity and protects the cell from mechanical damage, especially in the suspension cell culture. The trace elements and ions, such as SeO_3 and Selenium, play very important role in metabolic detoxification

2.1.3. Disadvantages of using serum in tissue culture

The composition of serum is complicated, including favorable components and unavoidable harmful ingredient. The disadvantages of using serum in tissue culture are listed as follow.

a. For most cells, serum is not physiological fluid in vivo. Cells only contact serum during the injury healing or blood coagulation. Thus the utilization of serum may change the normal condition of some cell. Serum may promote the growth of some cell (such as fibroblast) and inhibit the proliferation of other cells (such as epidermal kerotinocyte)
b. Some components may be toxic to cells. Take polyamine oxidase for example, it can react with the polyamine (such as spermine and spermidine) to form the toxic poly-spermine in highly proliferated cells. Furthermore, complements, antibodies and bacteriotoxin can affect the cell growth, or even lead to cell death.

c. Each batch of serum varies from others and the component can not maintain uniformity.
d. The productive process may infect with mycoplasma or virus, which potentially affect cells and lead to the fail experiment and unreliable experimental results.

2.2. Rat tail collagen

Rat tail collagen, used as either a thin layer on tissue-culture surfaces to enhance cell attachment and proliferation, or as a gel to promote expression of cell-specific morphology and function. This product is ideal for coating of surfaces, providing preparation of thin layers for culturing cells, or use as a solid gel. Rat Tail Collagen is suitable for applications using a variety of cell lines including hepatocytes, fibroblasts and epithelial cells

3. Synthetic medium

The synthetic medium is artificial designed and prepared medium. Nowadays the synthetic medium already becomes standardized commodity with wide varieties and convenience to use.

3.1. Basic medium

3.1.1. Basic components

After experimental selection, the simplest medium is minimum essential media (MEM), which contains more than 20 kinds of substance and can be divided to 4 subgroups.

Inorganic salt: $CaCl_2$, KCl, $MgSO_4$, NaCl, $NaHCO_3$, NaH_2PO_4

Amino acid: arginine, cystine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, histidine, tyrosine and valine.

Vitamins: partial polyoxometalates calcium, choline chloride, folic acid, inositol, nicotinamide, pyridoxine, riboflavin, thiamine

Carbohydrate: glucose

Besides the above substance related to cell growth, the medium usually also uses phenol red as pH indicator.

MEM is not common basic medium and common medium has more than 30 kinds of component, such as RPMI1640, DMEM. These mediums generally add some non-essential amino acids and vitamins, including serine, proline, biotin, Vitamin B12, etc.

3.2. Category

Nowadays, there are more than tens of basic medium available. The most common ones are listed as follow:

a. MEM

MEM, also called Eagle's minimal essential medium, is a cell culture medium developed by Harry Eagle that can be used to maintain cells in tissue culture. It only contains 12 kinds of non-essential animo acids, glutamine, 8 vitamins and some basic Inorganic salts

b. DMEM

A variation of MEM, called Dulbecco's modified Eagle's medium (DMEM), (Dulbecco/Vogt modified Eagle's minimal essential medium), contains approximately four times as much of the vitamins and amino acids present in the original formula and two to four times as much glucose. Additionally, it contains iron and phenol red. DMEM is further divide into high-glucose type (4500g/L glucose) and low-glucose type (1000g/L glucose). High-glucose DMEM is suitable for some tumor cells with faster growth speed and difficult attachment, as it is beneficial to retain and grow in one place.

c. IMDM

It is modified by Iscove basic DMEM and contains 42 ingredients. It includes selenium as well as additional amino acids and vitamins. In addition, this unique medium lacks iron, with potassium nitrate replacing ferric nitrate. It is well suited for difficult proliferating, low-density cell cultures, including hybrid cell selection after cell fusion, selection of transformed cell after DNA transfection.

d. RPMI1640

Roswell Park Memorial Institute medium, commonly referred to as RPMI, is a form of medium used in cell culture and tissue culture. The initial formula is suitable for growth of the suspension cells, mainly for lymphoid cells. This medium contains a great deal of phosphate and is formulated for use in a 5% carbon dioxide atmosphere. RPMI1640, the most mature improved medium, is suitable for most types of cells, including tumor cells, normal cells, primary culture cells, passage cell. RPMI1640 is one of the most common used medium.

e. 199/109 medium

199 medium is developed by Morgan and his coworkers in 1950 and is one of the earliest culture medium. It was originally developed as a completely defined media formulation for chick embryo cell culture. 199 medium has more than 60 components and contain almost all the amino acids, vitamins, growth hormone, nucleic acid derivative, etc. 109 medium is improved based on 199 medium and better formulated for the cell culture in a serum-free environment.

f. HamF10/HamF12

Ham's F-10 medium is a classical media designed by Ham to support the growth of mouse and human diploid cells in 1962. Ham's F-12, as improved products, has been used for the growth of primary rat hepatocytes and rat prostate epithelial cells. A clonal toxicity assay using CHO cells has also been reported with Ham's F-12 as the medium of choice. Ham's F-12 is also available with 25 mM HEPES buffer that provides more effective buffering in the optimum pH range of 7.2-7.4

g. McCoy's 5A

In 1959, McCoy and his coworkers reported the amino acid requirements for in vitro cultivation of Novikoff Hepatoma Cells. These studies were performed using Basal Medium 5A, and subsequently modified to create a new medium known as McCoy's 5A Medium. This media has been employed to support the growth of primary cultures derived from adrenal glands, bone marrow (normal), gingiva, lung, mouse kidney, omentum, skin, spleen and other tissues. It is a general purpose medium for primary and established cell lines.

All these medium are already commercialized and one medium have different forms, such as powder or liquid, large pack or pouch pack. The liquid form is subdivided 10x concentrated solution, 2x concentrated solution and working solution. Some medium don't have phenol red and some don't have calcium ion and magnesium. The user can choose product according to the experiment requirement. The basic components of partial medium are listed as follow:

Inorganic salts

Components	MEM	DMEM	IMDM	RPMI 1640	F10	F12	McCoys5A	199
$CaCl_2$	200.00	200.00	165.00	-	33.30	33.20	100.00	200.00
KCl	400.00	400.00	330.00	400.00	285.00	223.60	400.00	400.00
$MgSO_4$	98.00	97.67	98.00	48.84	74.60	-	98.00	98.00
NaCl	6800.00	6400.00	4500.00	6000.00	7400.00	7599.00	5100.00	6800.00
$NaHCO_3$	2200.00	3700.00	3024.00	2000.00	1200.00	1176.00	2200.00	2200.00
NaH_2PO_4	140.00	125.00	125.00	-	-	-	580.00	140.00
KNO_3	-	-	0.076	-	-	-	-	-
$NaSeO_3$	-	-	0.017	-	-	-	-	-
$Ca(NO_3)_2$	-	-	-	100.00	-	-	-	-
$CuSO_4$	-	-	-	800.00	153.70	142.00	-	-
Na_2HPO_4	-	-	-	-	0.03	0.86	-	-
$MgCl_2$	-	-	-	-	-	57.22	-	-
$Fe(NO_3)_3$	-	0.10	-	-	-	-	-	-
$CuSO_4$	-	-	-	-	0.0025	0.0025	-	-
$FeSO_4$	-	-	-	-	0.083	0.083	-	-
KH_2PO_4	-	-	-	-	83.00	-	-	-

Amino acids

Components	MEM	DMEM	IMDM	RPMI 1640	F10	F12	McCoys5A	199
L-ArginineHCl	126.00	84.00	84.00	200.00	211.00	211.00	42.10	70.00
L-Cystine2HCl	31.00	63.00	91.20	65.00	-	-	-	26.00
L-CystineHCl H_2O	-	-	-	-	25.00	35.00	31.50	0.10
L-HistidineHCl H_2O	42.00	42.00	42.00	15.00	23.00	21.00	21.00	22.00
L-Isoleucine	52.00	105.00	105.00	50.00	2.60	4.00	39.40	40.00
L-Leucine	52.00	105.00	105.00	50.00	13.00	13.00	39.40	60.00
L-LysineHCl	73.00	146.00	146.00	40.00	29.00	36.50	36.50	70.00
L-Methionine	15.00	30.00	30.00	15.00	4.50	4.50	15.00	15.00
L-Phenylalanine	32.00	66.00	66.00	15.00	5.00	5.00	16.50	25.00
L-Threomine	48.00	95.00	95.00	20.00	3.60	12.00	17.90	30.00
L-Tryptophan	10.00	16.00	16.00	5.00	0.60	2.00	3.10	10.00
L-Tyrosine2Na2H_2O	52.00	104.00	104.00	29.00	2.62	7.80	26.20	58.00
L-Valine	46.00	94.00	94.00	20.00	3.50	11.70	17.60	25.00
L-Alanine	-	-	25.00	-	9.00	8.90	13.90	25.00
L-Asparagine	-	-	25.00	50.00	15.00	15.00	45.00	-
L-Aspartic acid	-	-	30.00	20.00	13.00	13.00	20.00	30.00
L-Glutamic acid	-	-	75.00	20.00	14.70	14.70	22.10	75.00
L-Glutamine	-	584.00	284.00	300.00	146.00	146.00	219.20	100.00
Glycine	-	30.00	30.00	10.00	7.50	7.50	7.50	50.00
L-Proline	-	-	40.00	20.00	11.50	34.50	17.30	40.00
L-Serine	-	42.00	42.00	30.00	10.50	10.50	26.30	25.00
L-Hydroxyproline	-	-	-	20	-	-	19.70	10.00

Vitamins

Components	MEM	DMEM	IMDM	RPMI 1640	F10	F12	McCoys5A	199
K-Ca-Pantothenate	1.00	4.00	4.00	0.25	0.70	0.50	0.20	0.01
Choline Chloride	1.00	4.00	4.00	3.00	0.70	14.00	5.00	0.50
Folic acid	1.00	4.00	4.00	1.00	1.30	1.30	10.00	0.01
i-Inositol	2.00	7.20	7.20	35.00	0.50	18.00	36.00	0.05
Niacinamide	1.00	4.00	4.00	1.00	0.60	0.04	0.50	0.025
Pyridoxal HCl	1.00	-	4.00	-	-	-	0.50	0.025
Pyridoxine HCl	-	4.00	-	1.00	0.20	0.06	0.50	0.025
Riboflavin	0.10	0.40	0.40	0.20	0.40	0.04	0.20	0.01
Thiamine HCl	1.00	4.00	4.00	1.00	1.00	0.30	0.20	0.01
Biotin	-	-	0.013	0.20	0.024	0.007	0.20	0.01
Bitamin B_{12}	-	-	0.013	0.005	1.40	1.40	2.00	-
Para-aminobenzoic acid	-	-	-	1.00	-	-	1.00	0.05
Niacin	-	-	-	-	-	-	0.50	0.025
Ascorbic acid	-	-	-	-	-	-	0.50	0.05
a-Tocopherol phosphate	-	-	-	-	-	-	-	0.01
Calciferol	-	-	-	-	-	-	-	0.10
Menadione	-	-	-	-	-	-	-	0.01
Vitamin A	-	-	-	-	-	-	-	0.14

Other chemicals:

Components	MEM	DMEM	IMDM	RPMI 1640	F10	F12	McCoys5A	199
D-Glucose	1000.00	4500.00	44500.00	2000.00	1100.00	1802.00	3000.00	1000.00
Phenol red	10.00	15.00	15.00	5.00	1.20	1.20	10.00	20.00
HEPES	-	-	5958.00	-	-	-	5958.00	-
Sodium pyruvate	-	-	110.00	-	110.00	110.00	-	-
Glutathione(reduced)	-	-	-	1.00	-	-	0.50	0.05
Hypoxanthine.Na	-	-	-	-	4.70	4.77	-	0.04
Thymidine	-	-	-	-	0.70	0.70	-	-
Lipoic acid	-	-	-	-	0.20	0.21	-	-
Linoleic acid	-	-	-	-	-	0.08	-	-
Putrescine 2HCl	-	-	-	-	-	0.16	-	-
Bacto-peptone	-	-	-	-	-	-	600.00	-
Thymine	-	-	-	-	-	-	-	0.30

Adenine sulphate	-	-	-	-	-	-	-	10.00
Adenosine-5-triphosphate	-	-	-	-	-	-	-	0.20
Cholesterol	-	-	-	-	-	-	-	0.20
2-deoxy-D-ribose	-	-	-	-	-	-	-	0.50
Adenosine-5-phosphate	-	-	-	-	-	-	-	0.20
Guanine HCl	-	-	-	-	-	-	-	0.30
Ribose	-	-	-	-	-	-	-	0.50
Sodium acetate	-	-	-	-	-	-	-	50.00
Tween 80	-	-	-	-	-	-	-	20.00
Uracil	-	-	-	-	-	-	-	0.30
Xanthine Na	-	-	-	-	-	-	-	0.34

Table 2. The basic components of some medium (mg/L)

3.3. Serum-free medium

Serum free media (SFM) are important tools that allow researchers to grow a specific cell type or perform a specific application in the absence of serum.

3.3.1. Advantages of using serum free media include:

a. Easier purification and downstream processing
b. Precise evaluations of cellular function
c. Increased definition
d. More consistent performance
e. Increased growth and/or productivity
f. Better control over physiological responsiveness
g. Enhanced detection of cellular mediators

3.3.2. Things to consider in serum-free culture

Overall, cells in serum-free culture are more sensitive to extremes of pH, temperature, osmolality, mechanical forces, and enzyme treatment.

a. Antibiotics

It is best not to use antibiotics in serum-free media. If you do, we recommend that you use 5- to 10-fold less than you would in a serum- supplemented medium. This is because serum proteins tend to bind a certain amount of the antibiotic added; without these serum proteins the level of antibiotic may be toxic to certain cells.

b. Higher density

Cells must be in the mid-logarithmic phase of growth with viability >90% prior to adaptation. Sequential adaptation may be necessary.

Seeding cultures at a higher density than normal at each passage during SFM adaptation may help the process. Because some percentage of cells may not survive in the new culture environment, having more cells present will increase the number of viable cells to further passage.

c. Clumping

Cell clumping often occurs during adaptation to SFM. We recommend that you gently triturate the clumps to break them up when passaging cells.

d. Morphology

It is not uncommon to see slight changes in cellular morphology during and after adaptation to SFM. As long as doubling times and viability remain good, slight changes in morphology should not be a reason for concern.

3.4. Basic formula of serum-free medium

SFM includes basic culture medium and supplements. Basic culture mediums commonly use HamF12 and DMEM as 1:1 mixture. The supplements include:

a. Promote adherence substances

Many cells cultured in vitro have to attach the culture vessel for growth and SFM need to add some supplements for promoting attachment and extension, mainly extracellular matrix (fibronection, laminin, etc).

b. Somatomedin and hormones

Different growth factors need to add different cells. For example, EGF for the Keratinocytes, NGF for the Neurocyte, ECGF for the endothelial cell. The following table summarizes the utilization of some growth factor.

c. Enzyme inhibitor

Adherent cell need to trypsinize and passage. The SFM must have enzyme inhibitor to stop the activity of trypsin and protect the cell. The commonest enzyme inhibitor is soybean trypsin inhibitor

d. Binding protein(s) and translocator

The common binding protein and translocators are transferring and bovine serum albumin.

e. trace element

Selenium is most common trace element.

4. How to use

Sequential adaptation is preferred method for adapting cells to serum-free media (SFM).Because the change from 75% to 100% SFM may be too stressful for your cells, you

may need to carry the cells for 2–3 passages in a 10% serum-supplemented medium: 90% SFM mixture. Most cell lines can be considered fully adapted after 3 passages in 100% SFM. Occasionally you may have trouble getting your cells past a certain step even before going 100% SFM. If this happens, go back and passage the cells 2–3 times in the previous ratio of serum-supplemented media to serum-free media.

Growth factor	Target cell(common)	Target cell(specific)	Recommended concentration
EGF	epiblast, mesoblastema	Keratinocytes, fibroblast, chondrocyte, etc	1-20ng/ml
bFGF	Mesoblastema, neuroectoderm	endothelial cell, fibroblast, chondrocyte, myoblast,etc	0.5-10ng/ml
FGF	Mesoblastema, neuroectoderm	fibroblast, vascular cells.	1-100 ng/ml
ECGF	endothelial cell	endothelial cell	1-3mg/ml
IGF-1	Most cells		1-10ng/ml
PDGF	mesenchymal cell	fibroblast, myocyte, neurogliocyte	1-50ng/ml
NGF	sensory cell, sympathetoblast	Neurocyte, neurogliocyte	5-100ng/ml
TGF-α	Stimulate mesenchymal cell		0.1-3ng/ml
TGF-β	Inhibit epiblast		

Table 3. The utilization of some growth factor

4.1. Protein free medium(PFM) and chemical defined medium(CDM)

Protein free medium (PFM) is a proprietary serum-free and protein-free growth medium that does not contain any hormones or growth factors. This medium is optimized for the cultivation of Chinese Hamster Ovary (CHO) cells in addition to many derivatives of this parent line. The absence of protein in the medium eliminates any transmission risk of blood borne diseases, resulting in a product that is far safer than the current culture media on the market.

Chemical defined medium (CDM) is a media in which the chemical nature of all the ingredients and their amounts are known.

5. Other medium

5.1. Balanced salt solution (BSS)

Balanced salt solutions can provide an environment that maintains the structural and physiological integrity of cells in vitro. Solutions most commonly include sodium, potassium, calcium, magnesium, and chloride. 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. The following table lists the formula of several common BSS.

	Ringer	PBS	Tyrode	Earle	Hank's	D-Hank's	Dulbecco
NaCl	9.00	8.00	8.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.20	0.40	0.40	0.40	0.20
$CaCl_2$	0.25	-	0.20	0.20	0.14	-	0.10
$MgCl_26H_2O$	-	-	0.10	-	-	-	0.10
$MgSO_47H_2O$	-	-	-	0.20	0.20	-	-
$Na_2HPO_4H_2O$	-	1.56	-	-	0.06	0.06	-
$NaH_2PO_42H_2O$	-	-	0.05	0.14	-	-	1.42
KH_2PO_4	-	0.20	-	-	0.06	0.06	0.20
$NaHCO_3$	-	-	1.00	2.20	0.35	0.35	-
Glucose	-	-	1.00	1.00	1.00	-	-
Phenol red	-	-	-	0.02	0.02	0.02	0.02

Table 4. The formula of several common BSS

5.2. Medium used for digestion

Primary cell cultures usually need to digest and dissociate the tissue to cell suspension. Continuous passage cultures need to digest the adherent cell from culture dishes. The common digestion solutions are trypsin, EDTA solution and collagenase solution.

5.2.1. Trypsin

In a tissue culture lab, trypsins are used to re-suspend cells adherent to the cell culture dish wall during the process of harvesting cells. Trypsin is used to cleave proteins bonding the cultured cells to the dish, so that the cells can be suspended in fresh solution and transferred to fresh dishes.

Trypsin is an endopeptidase produced by the gastro-intestines of mammals, and has an optimal operating pH of about 8 and an optimal operating temperature of about 37 °C. The biochemical assays performed on Trypsin 1:250 determine both trypsin specific-activity at the level of certain co-purified enzymes that influence cell removal and viability

5.2.2. EDTA solution

In Cell Culture applications, EDTA is used for its chelating properties which binds to calcium and other ions and thus prevents adjoining of cadherins(i.e. the integral membrane proteins involved in calcium-dependent cell-adhesion) between cells, preventing the clumping of cells growing in liquid suspension or even detaching adherent cells for passaging. The working concentration is 0.02%. Trypsin//EDTA is a combined method for detaching cells.

5.2.3. Collagenase solution

Collagenase is especially valuable when tissues are too fibrous or too sensitive to allow the use of trypsin, which is ineffective on fibrous material and damaging to sensitive material. Dissociation is usually achieved either by perfusing whole organs or by incubating smaller pieces of tissue with enzyme solution. The working solution is 0.1-0.3mg/ml or 200000U/L. The optimal operating pH is about 6.5.

5.3. pH adjusting medium

5.3.1. $NaHCO_3$ solution

Sodium bicarbonate is used as part of a buffering system commonly used to maintain physiological pH 7.2 - 7.4 in a culture environment. The concentration of the sodium bicarbonate in the medium must be matched with the level of CO_2 in the atmosphere above the medium. For media containing 1.5 to 2.2 g/L sodium bicarbonate, use 5% CO_2. For media containing 3.7 g/L sodium bicarbonate, use 10% CO_2.

5.3.2. HEPES

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) is a zwitterionic organic chemical buffering agent. The level of HEPES in cell culture media may vary from 10mM to 25mM. It is added to the media solely for extra buffering capacity when cell culture requires extended periods of manipulation outside of a CO_2 incubator.

5.4. Antibiotics

Researchers use antibiotics and antimycotics to prevent contamination. The common ones are Penicillin & Streptomycin (P/S). Penicillin Streptomycin mixtures contain 5,000 units of penicillin (base) and 5,000 μg of streptomycin (base)/ml utilizing penicillin G (sodium salt)

and streptomycin sulfate in 0.85% saline. The following table summarizes the utilization of different antibiotics.

Antibiotic	Storage Conditions	Working Concentration	Stability in Culture (37°C)
Amphotericin B	-20°C	2.5 μg/ml	~3 days
Ampicillin	4°C	100 μg/ml	~3 days
Chloramphenicol	-20°C	5 μg/ml	~5 days
Ciprofoxacin	-20°C	10 μg/ml	not known
Gentamicin	4°C	50 μg/ml	~5 days
Hygromycin B	4°C	500 μg/ml	not known
Kanamycin	-20°C	100 μg/ml	~5 days
Neomycin	-20°C	50 μg/ml	~5 days
Penicillin	-20°C	100 U/ml	~3 days
Puromycin	-20°C	20 μg/ml	not known
Streptomycin	-20°C	100 μg/ml	~3 days
Tetracycline	-20°C	10 μg/ml	~4 days

Table 5. Antibiotics Storage Conditions Working Concentration Stability in Culture (37°C)

5.5. Glutamine

Glutamine is an unstable essential amino acid required in cell culture media formulations. Most commercially available media are formulated with free L-glutamine which is either included in the basal formula or added to liquid formulations at time of use. The concentration of L-glutamine used in classical media is usually 0.002mol/L.

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