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XerumFree™ XF212 Medium Supplement

Subject: Primary Cell Cultures

PRIMARY CULTURES

Primary cell cultures consist in growing cells immediately after their isolation from a living tissue or organism. They represent the core of the cell culture world: all existing cell lines to date have been initiated as primary cultures and this paradigm is here to stay. But apart from generating new cell lines, primary cultures represent also a very important tool by themselves, especially in fields such as drug discovery and development, regenerative medicine and fundamental research.

From a technical point of view, primary cell cultures remain also the most delicate part in the cell culture process. Freshly isolated cells have not been submitted to any selective pressure and remain highly representative of their in vivo counterpart. This fact calls for the outmost attention to satisfy their nutritional and physiological requirements. Ideally, the cell culture environment for primary cultures should mirror as closely as possible the in vivo situation, i.e. the extracellular space. This can only be achieved in defined cell culture conditions that allow for full control of the supply of nutrients and growth factors.

Primary cell cultures have highly disparate requirements, depending on their tissue of origin. XerumFree™ has proven successful in growing various primary cell cultures in the absence of undefined additions such as bovine serum or its derivates. There is however no universal recipe that would satisfy the needs of all primary cell types.

The following guidelines fall into two parts: general recommendations that apply to all cell types and specific requirements for the main tissue types.

AMSBIO | www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Primary Cell Cultures

COMMON RECOMMENDATIONS

When opting for a serum-free primary cell culture process the following points need to be addressed, no matter which cell type is used.

ABSENCE OF SERUM ATTACHMENT FACTORS

Preliminary step - Coating of the culture surface

In defined culture conditions, the treatment of the culture surface with an adequate coating strategy is of crucial importance. Usually crude preparations of extracellular matrices (ECM), such as mouse sarcoma extracts (e.g. matrigel) or extracted collagen preparations are commonly used. However, the undefined nature as well as the presence of animal-derived compounds renders their use problematic for many applications.

In the case that a contact with animal-derived material does not pose a problem, a 'quick-fix method' consisting of an overnight treatment of the plastic cell culture surfaces with a small amount of FBS may be considered. This method is cost convenient and efficient, however it represents a back step from the fully defined culture environment concept.

Today recombinant and defined coating kits are available, that mimic the attachment properties of ECM proteins through the use of biosynthetic signaling peptides derived from fibronectin, laminin, collagen, E- cadherin, vitronectin etc.

ABSENCE OF ENZYME INHIBITORS FROM SERUM

Dissociation enzyme

There are essentially two ways to start a primary culture: by outgrowth from a primary explant or by enzymatic disaggregation. In the latter method the starting tissue is digested by using proteolytic enzymes or cocktails of enzymes, such as dispase, collagenase and trypsin.

Care must be taken to neutralize / deactivate any remaining proteolytic activity before seeding the cells. This point must be addressed in particular when trypsin is employed.

The use of standard trypsin preparations can become problematic in the absence of serum, which contains trypsin inhibitors. In serum-deprived conditions the tryptic activity must be inactivated after the cell dissociation process, this can be achieved by using an efficient trypsin inhibitor, such as a soybean trypsin inhibitor.

As an alternative to trypsin, the use of Accutase™ is highly recommended because it does not need to be de-activated. This recombinant non-mammalian enzyme has been efficiently used for a series of primary cultures, including primary smooth muscle cells, primary human endothelial cells, primary chick neuronal cells.

ABSENCE OF BINDING BY SERUM PROTEINS

Use of Antibiotics

Antibiotics like many other compounds bind to the plasma proteins of serum, in particular to the albumin fraction. Thus, the same concentration of antibiotics will exhibit a much higher biological activity in serum- and albumin-free conditions and this increased activity may have deleterious impacts on cell growth. This is particularly the case for streptomycin which is known to interfere at the level of protein synthesis in mammalian cells.

In case 'antibiotic-free culture' is deemed unworkable, the use of gentamycin is suggested at the concentration of 50 mg/l.

Primary Cell Cultures

Celltype-specific recommendations

Primary cell cultures have different cell culture requirements, depending on their tissue of origin.

In this booklet we will not detail the primary cell culture procedures that differ vastly from one cell type to the other. As a general rule we recommend to apply the 'conventional' techniques for the isolation of the primary cells of the desired type and REPLACE the serum contribution by adding XerumFree™.

This will satisfy the nutritional requirements of most, if not all cell types. Indeed, in the realm of mammalian cell cultures, nutritional requirements vary only slightly quality-wise, more demanding cell types, such as hepatocytes requiring higher nutrient concentrations.

The growth factor and hormone requirements however differ between cell types. The following table lists the cell culture media preparations that we recommend when XerumFree™ is used as a replacement for animal serum.

The growth factor and hormonal additions are those indicated for optimal cellular development and proliferation with respect to each of the indicated cell types.

Recommended cell culture media set-up for a few major primary cell culture types

PRIMARY CELL CULTURE TYPE	RECOMMENDED GROWTH FACTOR	RECOMMENDED HORMONES	FINAL MEDIUM CONCENTRATION	REQUIREMENT
Primary kidney cultures				
XerumFree™ XF212 10%		Insulin (recombinant human)	0.5 µg/ml	essential
basal medium: DMEM high glucose / F-12 or Renal Epithelial Cell Basal Medium e.g. ATCC® PCS-400-030		Hydrocortisone	0.1 µg/ml	essential
		Epinephrine	0.5 µg/ml	essential
	EGF (human, recombinant)		50 ng/ml	optimal/ beneficial
		Triiodo-L- thyronine	10 pg/ml	essential
	EGF (human, recombinant)		10 ng/ml	optimal/ beneficial
Primary hepatocytes				
XerumFree™ XF212 10-15%		Insulin (recombinant human)	5 µg/ml	essential
basal medium: Williams' Medium E		Hydrocortisone	0.5 µg/ml	essential
	EGF (human, recombinant)		50 ng/ml	optimal/ beneficial
Primary keratinocytes*				
XerumFree™ XF212 10%		Bovine Pituitary Extract (BPE)	4 µl/ml	essential
DMEM/F-12 1:3 ratio		Hydrocortisone	5 µg/ml	essential
		Epinephrine	0.5 µg/ml	essential
	EGF (human, recombinant)		0.125 ng/ml	optimal/ beneficial
Primary cardiomyocytes				
XerumFree™ XF212 10%		T3 (triodo-L-thyronine)	1 ng/ml (1.5 nM)	essential
Claycomb Medium		Insulin (recombinant human)	5 µg/ml	essential
	EGF (human, recombinant)		5 ng / ml	optimal/ beneficial
	bFGF (human, recombinant)		5 ng / ml	optimal/ beneficial
Neuronal Cells				
XerumFree™ XF212 10%	EGF (human, recombinant)		50 ng/ml	optimal/ beneficial
DMEM high glucose		Insulin (recombinant human)	0.5 ug/ml	essential

* For correct cell attachment and spreading, addition of $CaCl_2$ (0.06 mM) is also highly recommended.

Nb. For additional types of primary cell culture types we invite you to contact AMSBio and we will be glad to supply our best possible support in terms of suggested growth factor and hormonal additions.

Nb. Since BPE ,Bovine Pituitary Extract, is from animal origin, one might consider to replace BPE by some recombinant aFGF and bFGF together with some heparin.

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F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85