Common Recommendations for Primary Cell Cultures

The following application note pertains to a variety of cell types used for serum-free primary cell culture methods.

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 crucial. Crude preparations of extracellular matrices (ECM), such as mouse sarcoma extracts (e.g. matrigel) or extracted collagen preparations are commonly used to coat culture surfaces. However, the undefined nature, as well as the presence of animal-derived compounds, renders their use problematic for many applications. If animal-derived material will not pose a problem, an overnight treatment of the plastic cell culture surfaces with a small amount of FBS may be considered as a 'quick-fix'. This method is cost-effective and efficient, but represents a step back 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 methods for initiating primary cultures: outgrowth from a primary explant or via enzymatic disaggregation. In the latter method, the starting tissue is digested using proteolytic enzyme cocktails, such as dispase, collagenase and trypsin. Care must be taken to neutralize/deactivate any remaining proteolytic activity before seeding the cells, particularly 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, tryptic activity must be inactivated after the cell dissociation process. This can be achieved by using an efficient trypsin inhibitor, such as soybean trypsin inhibitor.

As an alternative to trypsin, the use of Accutase™ is highly recommended because it does not require deactivation. This recombinant, non-mammalian enzyme has been efficiently used for multiple types of primary cultures, including primary smooth muscle cells, primary human endothelial cells, and 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, resulting in deleterious implications to cell growth. Streptomycin is particularly deleterious, as it is known to interfere at the level of protein synthesis in mammalian cells. In instances where 'antibiotic-free culture' is deemed unworkable, the use of gentamicin is suggested at a concentration of 50 mg/L.

Cell-type-specific Recommendations

Primary cell cultures have various requirements, depending on the tissue of origin. For the purposes of this application note, we will not discuss the primary cell culture procedures that differ vastly from one cell type to the other. Generally, we recommend applying the 'conventional' techniques for the isolation of primary cells of the desired type, replacing the serum source with the addition of FastGro. This will satisfy the nutritional requirements of most, if not all cell types. For the majority of mammalian cell cultures, nutritional requirements vary only slightly quantity-wise. More demanding cell types, such as hepatocytes, require higher nutrient concentrations.

Growth factor and hormone requirements often differ significantly between cell types. The table below lists the cell culture media preparations that we recommend when FastGro is used as a replacement for animal serum. The growth factor and hormone concentrations indicated are recommended for optimal cellular development and proliferation with respect to each of the indicated cell types.

Recommended cell culture media set-up using FastGro™ for select major primary cell culture types

PRIMARY CELL CULTURE TYPE	FASTGRO CONCENTRATION	RECOMMENDED BASAL MEDIUM	RECOMMENDED GROWTH FACTOR, FINAL CONCENTRATIONS & REQUIREMENT	RECOMMENDED HORMONES, FINAL CONCENTRATIONS & REQUIREMENT
Primary Kidney Cultures	FastGro™ 10%	DMEM high glucose / F-12	EGF (human, recombinant), 50 ng/mL, optimal/ beneficial	Insulin (recombinant human), 0.5 µg/mL, essential
				Hydrocortisone, 0.1 µg/mL, essential
				Triiodo-L- thyronine, 10 pg/mL, essential
				Epinephrine, 0.5 µg/mL, essential
Primary Hepatocytes	FastGro™ 10-15%	Williams' Medium E	EGF (human, recombinant), 50 ng/mL, optimal/ beneficial	Insulin (recombinant human), 5 µg/mL, essential
				Hydrocortisone, 0.5 µg/mL, essential
Primary Keratinocytes*	FastGro™ 10%	DMEM/F-12 (1:3 ratio)	EGF (human, recombinant), 0.125 ng/mL, optimal/ beneficial	Bovine Pituitary Extract (BPE), 4 µL/mL, essential
				Hydrocortisone, 5 µg/mL, essential
				Epinephrine, 0.5 µg/mL, essential
Primary Cardiomyocytes	FastGro™ 10%	Claycomb Medium	EGF (human, recombinant), 5 ng /mL, optimal/ beneficial	T3 (triodo-L-thyronine), 1 ng/mL (1.5 nM), essential
			bFGF (human, recombinant), 5 ng /mL, optimal/ beneficial	Insulin (recombinant human), 5 µg/mL, essential
Neuronal Cells	FastGro™ 10%	DMEM high glucose	EGF (human, recombinant), 50 ng/mL, optimal/ beneficial	Insulin (recombinant human), 0.5 µg/mL, essential

Recommendations for optimal cell culture performance with use of FastGro:

- As a basal medium, select DMEM/F12, Williams' medium E or Ham's F12
- Avoid the use of antibiotics, as FastGro lacks albumins, and all added antibiotics will remain free and active, increasing risk of cytotoxicity. If antibiotics must be used, choose gentamicin over pen-strep.
- We advise utilizing a gentle dissociation reagent for cell detachment, such as Accutase, while avoiding the use of trypsin. Since Accutase is an enzyme derived from tropical shellfish, it works optimally at 25°C, but decays quickly at higher temperatures.
- When refreshing the medium, we advise retaining approximately 25% old (conditioned) medium and adding 75% fresh prepared medium. Valuable factors produced and excreted by the cultured cells can be collected, filtered, and frozen from old media for future use.

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