FREQUENTLY ASKED QUESTIONS

1. What is City-mix™?

City-mix™ is our range of proprietary media supplements specially designed to boost cell growth and tissue production in a wide variety of culture conditions. City-mix™ is a chemically-defined solution comprising highly stable and inert, food safe, ingredients that enhance the chemical activity of most components found in common culture media (e.g., albumin, growth factors, nutrients, enzymes, etc) via volume exclusion mechanisms. City-mix™ is not a complete cocktail of growth factors, but works by reducing or eliminating growth factor supplementation in culture media.

2. Is City-mix™ suitable for all cell lines?

City-mix™ effectively increases proliferation rates and/or extracellular matrix establishment across all the cell lines we have tested it on. However we have found that certain cells perform better with different City-mix™ components. At 3D Bio-tissues we have optimised our formulations across multiple cell lines which has resulted in different City-mix™ products outlined below:

City-Mix™ supplements overview	Application
Shanghai-Mix™	
Our most versatile cell booster for enhanced cell and tissue growth, effective in both serum-free or serum-containing media	Myoblasts, adipocytes, fibroblasts
Singapore-Mix™	
Another good all-rounder, particularly effective for enhanced fat cell growth and for increased production of connective tissue	Adipocytes, myoblasts, fibroblasts
Tokyo-Mix™	
Low-viscosity booster, and particularly effective for enhanced stem cell proliferation	ASC/MSCs
London-Mix™	
Our best supplement for enhanced extracellular matrix production	Fibroblasts, myoblasts, adipocytes
Miami-Mix™	
A cell booster useful to control spontaneous cell differentiation occurring in serum-free conditions	Myoblasts, adipocytes, stromal cells

3. Is City-mix™ a direct serum replacement?

City-mix™ is not a direct serum replacement, and does not contain the nutrients and growth factors commonly found in animal serum. Media supplemented with City-mix™ will still need to include specific growth factors to be effective, but at much lower concentrations than typically used.

In all biologic systems, cell and tissue growth depends on the bioavailability of nutrients and growth factors. Ultimately, the type and quantity of raw ingredients comprising a given culture medium determine how cells behave in vitro. *For example, albumins, insulin, and vitamin C are key components of most serum-free culture media and must be present in formulations supporting the proliferation of both primary and immortalised cells. Albumins are large, highly abundant antioxidant proteins whose primary function is to bind, sequester and stabilise a range of small molecules and ions. Insulin plays a fundamental role in glucose and amino acid uptake, and cell cycle progression. Vitamin C derivatives regulate extracellular matrix production, and without them cells fail to express many of its components. Without these "raw ingredients" in the media, cells cannot grow or develop.*

This means that City-mix™ can effectively reduce the amount of many of the essential growth factors necessary to sustain specific culture processes such as cell proliferation and tissue maturation. *City-mix™ will make these expensive media ingredients go much further via volume exclusion effects. In particular, using City-mix™ to reduce albumin supplementation allows for chemically-defined, xeno-free medium formulations to become cost-effective.*

4. Is City-mix™ food safe?

The ingredients of City-mix™ are inert, non-toxic (zero toxicity), food safe, high molecular weight molecules that are widespread in the food and cosmetic industry worldwide.

5. Does City-mix™ feed cells?

We have no evidence that cells can extract any nutritional value from City-mix™. Its high stability in culture also suggests that City-mix™ ingredients are not consumed by cells.

6. What growth factors should I use?

Serum Free Media formulations vary significantly depending on application and cell line. Common growth factor supplements outlined in the literature include:

- *Insulin*
- *IGF-1*
- *FGF2*
- *TGFβ*
- *VEGF*
- *HGF*
- *PDGF-BB*
- *Albumin*
- *Transferrin*
- *Vitamins, e.g. ascorbic acid*

7. **If City-mix™ increases extracellular matrix production, will that make cells grown in suspension to aggregate?**

   City-mix™ promotes extracellular matrix production indirectly by enhancing the chemical activity of other media ingredients that actively regulate matrix biosynthesis. If your suspension formulation does not contain such ingredients (e.g., sodium ascorbate), then City-mix™ is not expected to substantially increase cell aggregation.

8. **How viscous is City-mix™, and will this affect cultures in suspension?**

   At our recommended effective concentrations, the viscosity of City-mix™ is comparable to that of animal serum, and is therefore expected to affect suspension cultures to a similar degree. For cultures where even small increases in medium viscosity are to be avoided, we recommend using our low-viscosity supplement, Tokyo-Mix™.