# FREQUENTLY ASKED QUESTIONS

## 1. What is CytoBoost?

*CytoBoost is our premium "first-aid kit" for your cells, specially formulated to optimise cell health and performance. CytoBoost cell care comprises a tailored range of chemically defined, stable, non-toxic, and inert macromolecules that enhance the chemical activity of culture media components (e.g., albumins & globulins, growth factors, macro and micro nutrients, enzymes, etc) via volume exclusion mechanisms. Adding a small amount of a CytoBoost product to your media can offer the following benefits:*

- *Reduces the reliance on growth factors in media*
- *Minimises serum requirements in media*
- *Enhances cell viability post-thawing*
- *Improves overall cell performance*
- *Promotes tissue formation and cell proliferation*
- *Increases production, quality, and stability of biologics, including hormones, growth factors, peptides/polypeptides, viral particles/vaccines, antibodies, interferons, interleukins, and exosomes*

*CytoBoost is not a combination of growth factors, but works by improving the biochemical and biophysical environment of the culture medium surrounding cells.*

## 2. Is CytoBoost suitable for all cell types?

*CytoBoost is suitable for all cell types and all animal species, including human cells. CytoBoost has been successfully tested with multiple cell types, including but not limited to:*

- *Adult mesenchymal stem cells*
- *Induced pluripotent stem cells*
- *Embryonic stem cells*
- *Primary and immortalised fibroblasts*
- *Primary and immortalised myoblasts*
- *Primary and immortalised adipocytes*

*In addition, CytoBoost Vision was specifically developed to enhance the performance of corneal stromal cells.*

## 3. Is CytoBoost a direct serum replacement?

*CytoBoost is not a direct serum replacement, and does not contain any serum ingredients. Media with a CytoBoost additive will still need to include traditional media bio-active components, but often at much lower concentrations than typically used.*

*More information can be found in our Application Note: How Macromolecular Crowders can Boost Animal Cell Culture.*

*Version: 1.1*

4. **Does CytoBoost feed cells?**

   *There is no evidence that cells extract nutritional value from CytoBoost. Furthermore, retention analysis studies have shown that CytoBoost Develop, Maximise and Perform are not uptaken by cells, even when used in high concentrations. As such, please ensure that your culture medium of choice contains all the nutrients essential to support cell growth before adding CytoBoost.*

5. **What growth factors should I use?**

   *Media formulations vary significantly depending on application and cell type. CytoBoost is a media additive which does not contain additional growth factors, so make sure that media supplemented with CytoBoost already contains all the growth factors essential to support your cell culture. However, certain formulations are used successfully to reduce the required concentration of growth factors by up to 75% without loss in cell performance*

6. **If CytoBoost increases extracellular matrix production, will that induce cells grown in suspension to aggregate?**

   *Certain CytoBoost formulations promote extracellular matrix production indirectly by enhancing the chemical activity of other media ingredients that actively regulate matrix biosynthesis. Its use can enhance the effects of other additives, thus under certain user defined media formulations this may well increase aggregation further.*

7. **How viscous is CytoBoost, and will this affect cultures in suspension?**

   *At our recommended effective concentrations, the viscosity of CytoBoost is comparable to that of animal serum, and is therefore expected to affect suspension cultures to a similar degree.*

8. **Is CytoBoost ready to use?**

   *Yes. As an additive, CytoBoost can be readily added to culture media for immediate use, usually at a 1-8% (v/v) concentration; ensure the CytoBoost solution is well mixed and homogeneous before pipetting and after being added to culture media. CytoBoost is prepared with the highest quality, research grade ingredients and provided as a sterile solution suitable for cell culture. Always open and use CytoBoost in aseptic conditions to avoid introducing contamination into your culture.*

9. **How should I store CytoBoost?**

   *CytoBoost products are shipped at room temperature; however, we recommend storage at 2-8°C away from sources of light to maximise shelf life, especially once opened. Warm CytoBoost to 37°C and invert bottles 5-10 times prior to use. As CytoBoost is sold as a sterile solution, filtering prior to use is not required; however you can filter culture media containing CytoBoost if convenient or necessary, as CytoBoost ingredients are not retained by most types of filter membranes.*

**10. How stable is CytoBoost?**

*CytoBoost is very stable and fully effective when handled and stored according to instructions and within its expiry date (which is 1 year from manufacture, as stated on the bottles). We do not expect the stability of CytoBoost to be affected by dilution after addition to culture media; warm media with added CytoBoost to 37°C and mix thoroughly before use.*

**11. When will I see the boosting effects of CytoBoost?**

*The volume exclusion effects of CytoBoost are observed immediately after mixing with culture media. However, its biological effects depend on cell type and metabolism, media composition, and the parameters being evaluated. For example, its effects on animal cell viability and proliferation can be quantified even after just one day in culture, whereas the impact on extracellular protein production may require longer culture periods.*

# TROUBLESHOOTING GUIDE

| Potential issues | Possible Causes | Suggested Solutions |
|---|---|---|
| Cells grow well in my base medium, but CytoBoost is not boosting the growth rate significantly | • Cell seeding density too high - cultures become confluent sooner, and contact inhibition and/or detachment leads to underestimated cell count<br>• Cell seeding density too low - sparse cells show sluggish initial growth, delaying boosting effects to later time points<br>• The expiry date of CytoBoost exceeded<br>• CytoBoost stored inadequately<br>• CytoBoost used at incorrect concentration (pipetting error, poor mixing of CytoBoost before or after being added to media)<br>• Medium supplemented with CytoBoost stored inadequately, or for too long, or not heated or mixed before use<br>• Growth factors in medium are already at optimal concentration | Try using Maximise or Perform as they work best with boosting growth rates.<br>Seed cells at different densities and/or monitor culture progression more frequently<br>Use fresh CytoBoost<br>Prepare fresh medium with CytoBoost at recommended concentrations<br>Use base medium containing 25-75% less growth factors<br>Try CytoBoost at a lower concentration - we suggest testing a range of concentrations between 2 and 10%. |
| Cells grow poorly in my base medium, and CytoBoost is not helping | • The concentration of growth factors in the medium is too low or nonexistent, CytoBoost is unable to boost their activity<br>• Insufficient cell adaptation to serum-free/reduced serum/ new base medium conditions<br>• CytoBoost used at incorrect concentration, not mixed sufficiently; supplemented medium stored inadequately or for too long | Use a base medium with growth factors appropriate for growing that specific cell type<br>Gradually reduce serum amount over multiple passages<br>Prepare fresh medium with CytoBoost at recommended concentrations |
| CytoBoost is having negative impact on cell growth | • CytoBoost being used at too high concentration<br>• Concentration of growth factors in medium are too high, CytoBoost is making them toxic<br>• Base medium not being used as positive control condition<br>• Base medium has one or more ingredients incompatible with that specific CytoBoost | Use lower amounts of CytoBoost<br>Use base medium containing 25-75% less growth factors<br>Use base medium as control to calculate baseline growth<br>Consider using a different CytoBoost formulation |

# TROUBLESHOOTING GUIDE

| Potential issues | Possible Causes | Suggested Solutions |
|---|---|---|
| Cells show low attachment, altered morphology and/or differentiation state | • Cells are nearing post-mitotic or senescence state, or showing phenotypic instability<br>• Cell viability after passage is low e.g. due to harshness of the process, anoikis, etc.<br>• Cells detach during proliferation<br>• Culture surface is not adequate for cell attachment; surface has been altered by manufacturer<br>• CytoBoost is promoting the activity of a cell differentiation factor present in the base medium | Replace cultures with lower passage cells<br>Use a gentler passaging process<br>Use cell-adhesive coatings<br>Change surface; use cell-adhesive coatings<br>Reduce concentration of specific differentiation factor by 25-75% |
| Results are unclear | • Cells growth is patchy/heterogeneous, with the presence of cell aggregates or clusters dispersed throughout the cell culture surface<br>• Experiment lacks appropriate positive and/or negative controls<br>• CytoBoost was membrane-filtered prior to use, resulting in losses of one or more of its ingredients, and in incorrect final concentration<br>• Evaluation may have been assessed at a wrong time point - with optimal results missed or yet to happen due to effects of CytoBoost<br>• Cell quantification assay not appropriate, or performed without appropriate positive and/or negative controls | Suspend cells homogeneously with CytoBoost -supplemented medium prior to seeding<br>Please refer to our Companion Guide for examples<br>CytoBoost is already sterile; if necessary, filter medium after CytoBoost supplementation<br>Plan experiments with wider range of time points, depending on parameters under analysis<br>Change quantification assay (e.g., direct cell counting instead of metabolic assay) |

# CytoBoost media supplements

xeno-free cell boosters

PRODUCT COMPANION GUIDE

## What is CytoBoost?

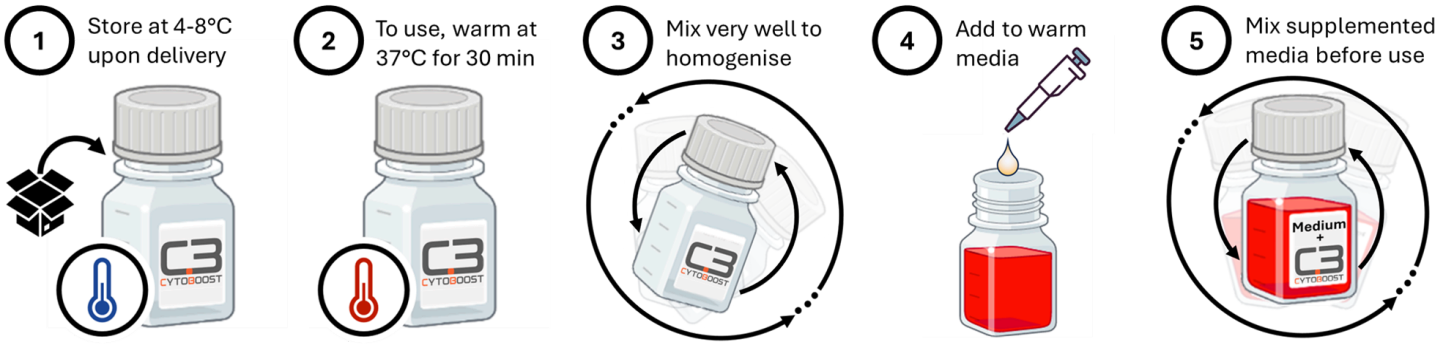
CytoBoost is a line of macromolecular crowders specifically designed as xeno-free media additives for enhancing cell performance, including in high-serum, reduced serum, and serum-free culture conditions.

CytoBoost has been carefully formulated to address a wide range of applications in multiple animal cells types (*e.g.*, stem cells, iPSCs, tumour cells, fibroblasts, CHO, Vero, Per.C6, HT1080, NS0, Sf9, *etc.*), with four offerings currently available:

- **Perform** - An outstanding all-rounder, delivering exceptional results across multiple applications.
- **Maximise** - Promotes cell proliferation in 2D and 3D cultures, ensuring rapid and efficient growth for superior experimental outcomes.
- **Revive** - Enhances cell recovery during freeze-thaw revival, significantly boosting cell viability.
- **Develop** - Promotes the formation of a strong and resilient extracellular matrix, supporting optimal cell growth and tissue formation.
- **Vision** - Specially designed to enhance the health and performance of corneal cells, optimising their viability and function

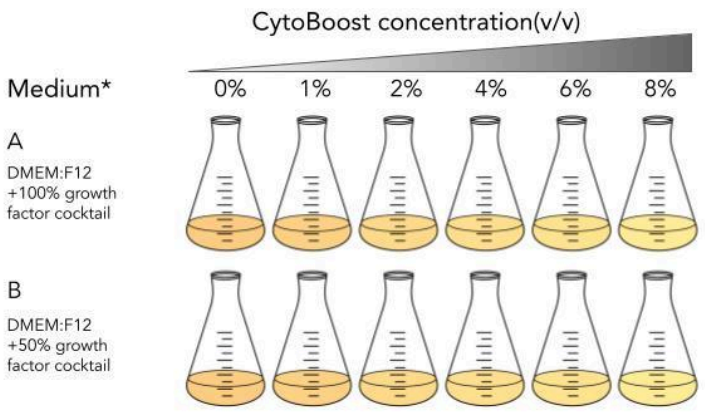
## How do I use CytoBoost?

CytoBoost is a cell culture additive, and as such should be added to your complete liquid media of choice.
Discover below the five quick and easy steps to add CytoBoost to your media and start boosting your cell culture!

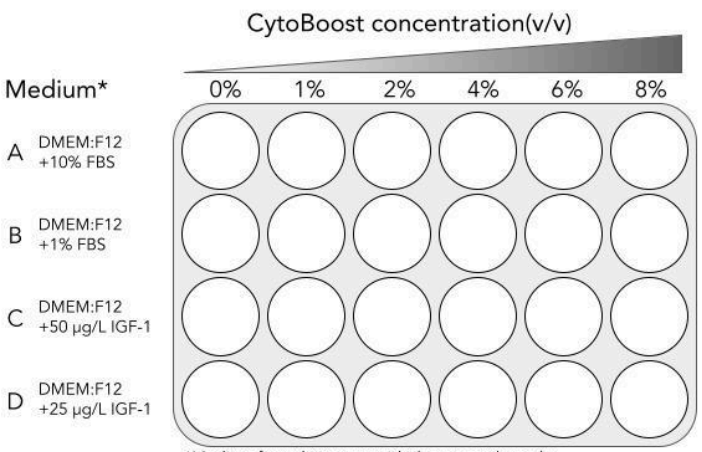



## How much CytoBoost should I add to my media?

The examples below illustrate quick and easy ways to start optimising CytoBoost concentration in your 2D or 3D cultures. Adding CytoBoost to media at a final concentration of 1 to 8% (v/v) leads to superior cell survival, growth, and/or productivity rates, and can help you get better results, consistently, reliably, and without extra effort!




*Medium formulations provided as examples only. Composition of growth factor cocktail: L-ascorbic acid 2-phosphate trisodium (1 mM), insulin (5 mg/L), transferrin (5 mg/L), sodium selenite (20 µg/L), FGF2-G3 (40 µg/L), TGFβ3 (100 ng/L), and NRG1 (100 ng/L).




*Medium formulations provided as examples only.