

**Supplementary Table S1:** Functions and recommended concentrations of various components.

Component	Function	Recommended Concentrations	References
ITS-X	ITS-X is listed as one of the common additives used to support cell metabolism and antioxidant function in serum-free media.	1 ×	[1-4]
BFGF	BFGF could promote cell growth, proliferation and wound healing.	1-200 ng/mL	[1,5-8]
BSA	BSA acts as a carrier protein that stabilizes growth factors and hormones in the medium while providing nutrients necessary for cell growth.	0.1-0.5%	[4,6,9]
Y-27632 2HCl	Y-27632 2HCl is a selective inhibitor of ROCK1 and ROCK2, and can reduce apoptosis induced by cell division.	2-10 µM	[8,10,11]
EGF	EGF can promote the proliferation of various cells, including muscle cells, epithelial cells, and endothelial cells.	2-200 ng/mL	[1,6,12,13]
IGF-1	IGF-1 promotes the proliferation and differentiation of skeletal muscle cells during myogenesis.	10-200 ng/mL	[1,12,14]
LIF	LIF can promote cell proliferation and maintenance.	0.1-50 ng/mL	[5,15,16]
Lipids concentration	Lipids play a vital role in serum-free media by serving as essential components for cell membrane structure, energy storage, and signaling pathways.	1 ×	[6]
NEAA	Non-essential amino acids play a significant role in serum-free cell culture by supporting cell growth, metabolism, and protein synthesis.	1 ×	[8]
L-Ascorbic acid	L-Ascorbic acid functions as an effective antioxidant and a cofactor for various enzymatic reactions. It helps mitigate oxidative stress, which is particularly important in the absence of serum-derived protective factors.	1-150 µM	[17,18]
Asc-2P	Asc-2P is a stable derivative of L-Ascorbic acid.	1-150 µM	[19]
Trolox	Trolox is a water-soluble derivative of vitamin E and plays a crucial role in serum-free cell culture by acting as a potent antioxidant.	50 µM	[20]
NAC	NAC acts as a precursor for glutathione synthesis, enhancing cellular antioxidant capacity to mitigate oxidative stress and protect cells from toxin-induced damage in serum-free culture systems.	1-5 mM	[20,21]
Hydrocortisone	Hydrocortisone can modulate glucocorticoid receptor activity and glucose metabolism to support cell survival under stress conditions.	100 nM	[1,6]
Forskolin	Forskolin elevates intracellular cAMP levels, promoting cell differentiation and autophagy, modulates metabolic pathways and receptor activity to support cell survival under stress conditions.	10-100 µM	[22]
HGF	HGF promotes cell survival, growth, motility and morphogenesis	0.1-50 ng/mL	[1,5]
Dexamethasone	Dexamethasone can regulate cell growth and proliferation, suppress inflammatory responses, and enhance cellular responsiveness to other stimuli.	10-500 nM	[1,23]
LPA	LPA enhances cell adhesion and migration, promotes proliferation, and inhibits apoptosis.	5 µM	[24,25]

## References

1. Kolkman AM, Van Essen A, Post MJ, Moutsatsou P. 2022. Development of a Chemically Defined Medium for in vitro Expansion of Primary Bovine Satellite Cells. *Frontiers in Bioengineering and Biotechnology* 10:895289
2. Chen L, Wang N, Zhang T, Zhang F, Zhang W, et al. 2024. Directed differentiation of pancreatic  $\delta$  cells from human pluripotent stem cells. *Nature Communications* 15:6344
3. Bayer Andersen K, Leander Johansen J, Hentzer M, Smith GP, Dietz GPH. 2016. Protection of Primary Dopaminergic Midbrain Neurons by GPR139 Agonists Supports Different Mechanisms of MPP<sup>+</sup> and Rotenone Toxicity. *Frontiers in Cellular Neuroscience* 10:164
4. Takii S, Wu J, Okamura D. 2022. The amount of membrane cholesterol required for robust cell adhesion and proliferation in serum-free condition. *PLoS One* 17:e0259482
5. Stout AJ, Mirliani AB, Rittenberg ML, Shub M, White EC, et al. 2022. Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat. *Communications Biology* 5:466
6. Devireddy LR, Myers M, Screven R, Liu Z, Boxer L. 2019. A serum-free medium formulation efficiently supports isolation and propagation of canine adipose-derived mesenchymal stem/stromal cells. *PLoS One* 14:e0210250
7. Yao S, Chen S, Clark J, Hao E, Beattie GM, et al. 2006. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *Proc Natl Acad Sci U S A* 103:6907-12
8. Liuyang S, Wang G, Wang Y, He H, Lyu Y, et al. 2023. Highly efficient and rapid generation of human pluripotent stem cells by chemical reprogramming. *Cell Stem Cell* 30:450-59.e9
9. Haredy AM, Takenaka N, Yamada H, Sakoda Y, Okamatsu M, et al. 2013. An MDCK cell culture-derived formalin-inactivated influenza virus whole-virion vaccine from an influenza virus library confers cross-protective immunity by intranasal administration in mice. *Clin Vaccine Immunol* 20:998-1007

10. Zhu G, Gao D, Li L, Yao Y, Wang Y, et al. 2023. Generation of three-dimensional meat-like tissue from stable pig epiblast stem cells. *Nat Commun* 14:8163
11. Hyvelin JM, Howell K, Nichol A, Costello CM, Preston RJ, McLoughlin P. 2005. Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation. *Circ Res* 97:185-91
12. Wu X, Kang H, Liu X, Gao J, Zhao K, Ma Z. 2016. Serum and xeno-free, chemically defined, no-plate-coating-based culture system for mesenchymal stromal cells from the umbilical cord. *Cell Prolif* 49:579-88
13. Garcez RC, Teixeira BL, dos Santos Schmitt S, Alvarez-Silva M, Trentin AG. 2009. Epidermal Growth Factor (EGF) Promotes the In Vitro Differentiation of Neural Crest Cells to Neurons and Melanocytes. *Cellular and Molecular Neurobiology* 29:1087-91
14. Lei Q, Li M, Du G, Zhou J, Guan X. 2022. An effective cytokine combination for ex vivo expansion of porcine muscle stem cells. *Food Bioscience* 46
15. Liu B, Chen S, Xu Y, Lyu Y, Wang J, et al. 2021. Chemically defined and xeno-free culture condition for human extended pluripotent stem cells. *Nat Commun* 12:3017
16. Wang X, Wu H, Zhang Z, Liu S, Yang J, et al. 2008. Effects of interleukin-6, leukemia inhibitory factor, and ciliary neurotrophic factor on the proliferation and differentiation of adult human myoblasts. *Cell Mol Neurobiol* 28:113-24
17. Moteki H, Shimamura Y, Kimura M, Ogihara M. 2012. Signal transduction pathway for L-ascorbic acid- and L-ascorbic acid 2-glucoside-induced DNA synthesis and cell proliferation in primary cultures of adult rat hepatocytes. *Eur J Pharmacol* 683:276-84
18. Yang M, Teng S, Ma C, Yu Y, Wang P, Yi C. 2018. Ascorbic acid inhibits senescence in mesenchymal stem cells through ROS and AKT/mTOR signaling. *Cytotechnology* 70:1301-13
19. Zhu H, Wu Z, Ding X, Post MJ, Guo R, et al. 2022. Production of cultured meat from pig muscle stem cells. *Biomaterials* 287:121650
20. Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, et al. 2009. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature* 461:109-13
21. Xue L, Li J, Li Y, Chu C, Xie G, et al. 2015. N-acetylcysteine protects Chinese Hamster ovary cells from oxidative injury and apoptosis induced by microcystin-LR. *Int J Clin Exp Med* 8:4911-21
22. Middleton P, Jaramillo F, Schuetze SM. 1986. Forskolin increases the rate of acetylcholine receptor desensitization at rat soleus endplates. *Proc Natl Acad Sci U S A* 83:4967-71
23. Liu Z, Screven R, Boxer L, Myers MJ, Devireddy LR. 2018. Characterization of Canine Adipose-Derived Mesenchymal Stromal/Stem Cells in Serum-Free Medium. *Tissue Eng Part C Methods* 24:399-411
24. Miki H, Takagi M. 2015. Design of serum-free medium for suspension culture of CHO cells on the basis of general commercial media. *Cytotechnology* 67:689-97
25. Xu J, Lai YJ, Lin WC, Lin FT. 2004. TRIP6 enhances lysophosphatidic acid-induced cell migration by interacting with the lysophosphatidic acid 2 receptor. *J Biol Chem* 279:10459-68