deAlbumin™

Solving Mysteries in Your Cell Culture





"Formulated" recombinant Human Serum Albumin

When serum albumin is isolated from serum or recombinant hosts, a handful of bioactive molecules are associated with it. These impurities of albumin cause its lot-to-lot performance variation.

We study the small molecule profile of albumin and formulate highly purified albumin accordingly, to reconstitute albumin's optimum performance for each cell type you wish to grow.

deAlbumin^ $\!\!\!^{\mathrm{TM}}$ products are tested with iPS, T-Cell, MSC and Neuron.

SKU	FORMAT	APPLICATION
DA01-20-25ML	20% DPBS Solution	MSC, Neuron
DA01-1G	Lyophilized	MSC, Neuron
DA07-20-25ML	20% DPBS Solution	T-cell, MSC, Neuron
DA06-10-25ML	10% DPBS Solution	iPS

Application: Healthier NEURON CULTURE



Culturing neuronal cells in vitro has been a model system for studying neurosciences, neuro diseases, and various aspects of drug developments. It is convenient, as researchers can visually access and manipulate the living cell.

For decades of effort, several methodologies of culturing from neuronal stem cells (NSC) to neuron subtypes are developed, and these NSC can either be derived from iPS, ES, or isolated from primary tissue.

Today, while commercial medium and supplements have claimed the opposite, many labs still prefer to apply astrocyte feeder in neuronal culture, aiming for the longevity of cell in the dish and based on the belief that cells are "healthier" in co-culture environment.

Albumin is used in most reported medium formula (ref. 1), and it is critical for the plating survival rate as well as the maturation of neurons (ref. 2, 3).

Here, as a supplement of feeder-free NSC culture, our deAlbuminTM, could yield a <u>higher survival rate</u> and <u>faster maturation</u> toward the co-culture system. In NS21/Neurobasal system, deAlbuminTM (DA01, DA07) increases the plating NSC cell number by 73% and the maturation of neuron which can be quantified by the 5-fold length of neurite outgrowth.

SPECIFICATION

Biological source	human, recombinant expressed in <i>Pichia pastorias</i>
Assay	≥98% Albumin, ≥90% monomer by PAGE
Mol. wt	monomer ~67 kDa
Endotoxin	<1 EU/mg protein
Storage temp.	2-8°C
Gene	ALB (Human)

Images





1. Brewer, G. J. et al. Journal of Neuroscience Research 35, 567-576 (1993).

- 2. Chen, Y. et al. Journal of Neuroscience Methods 171, 239-247 (2008).
- 3. Tabernero, A. et al. Journal of Neurochemistry 81, 881-891 (2002).

Figure 1. Maintenance of Rat Cortical Neuron. NS21 is prepared according to Ref. 2. Primary Rat Cortex neuronal cells (Cat. #A18945), B27 (Cat. #17504044) and Neurobasal (Cat #A3582901) are from ThermoFisher, BSA (Cat #A4919) is from Sigma Aldrich. Cortex neuronal cells are thaw and cultured according to ThermoFisher's manual, 5x10³ neurons / cm² are plated on 0 DIV and monitored daily.



Application: Robust Medium for PSC



Tweaking the composition of commercial ES or iPS medium is oftentimes necessary to suit each research project. However, sourcing customized medium from a vendor typically involve lengthily negotiation with media producers.

And how about brewing hES or hiPS media in house? One choice is to start from the Essential 8 medium (E8), and since its recipe is published in 2005 (ref 4.), one can tune the composition accordingly. However, A major issue with E8 medium is its robustness in the situation where cells are stressed. For example, one might find low yields in applications such as single cell cloning, patient-derived iPS, or CRISPR-Cas9 gene editing. Several growth factors, including albumin, can be added as a supplement to enhance the E8 medium, and they are mostly available as recombinant form and with high purity, except for albumin.



Recently, several advanced ES/iPS culturing medium containing albumin have emerged (ref. 5), and the possible mechanism of albumin in these products versus the E8 which lacks albumin is also reported (ref. 6)

We developed deAlbumin[™] (DA06) specifically for iPS cell, and it raises cell numbers in E8 culture by 45%.



- 4. Chen, G. et al. Nature Methods 8, 424-429 (2011).
- 5. Desai, N. et al. Reproductive Biology and Endocrinology 13, 9 (2015)
- 6. Massai, D. et al. Scientific Reports 7, (2017).

Figure 2. deAlbuminTM supports the growth and maintenance of iPSC. iPS cells (GIBCO, #A18945) were cultured in E8 medium (GIBCO, #A1517001) supplemented with each recombinant albumin (1 mg/mL). iPS cells were seeded at $1x10^{5}$ /well in 6-well plate. Medium was changed daily. After culturing for 4 days, the viable cell densities of each group were determined by trypan blue methods.

Application: Massive Expansion of T-CELL



T-cells and NK cells are used in many clinical trials as immunotherapy. Isolated from the pool of peripheral blood mononuclear cells (PBMC), they are oftentimes genetically modified, such as the installation of chimeric antigen receptors, to gain

additional functionalities.

Under the constrain of the finite lifespan of these primary cells (30-40 days for T cells and 15 days for NK cells, ref. 7 and ref. 8), it becomes necessary to optimize the culturing medium, so that the engineered and expanded cells are sufficient for therapeutic regiments.

A Serum-free culture system for T-cell and NK cell has been developed since 1987, and albumin was found to be an indispensable substitute component of serum to promote cell growth (ref. 9). Statistical medium optimization also identified albumin as critical factors (ref. 10).

The impurities on albumin would not only affect the lymphocyte cultures nutritionally but also possibly have proinflammatory or immunoregulatory effects (ref 11). Our DA07 albumin can be used in both static culture and shaking culture. In 5-day culture, with deAlbumin^M (DA07), the viable cell growth is increased by 99% comparing to CDM without albumin.



- 7. Baliu-Piqué, M. et al. Frontiers in Immunology 9, (2018).
- 8. Lowry, L. E. et al. Frontiers in Immunology 8, (2017).
- 9. Polet, H. et al. The Journal of Experimental Medicine 142, 949 (1975).
- 10. Kim, M. M. et al. Communications Biology 2, (2019).
- 11. Lone, A. M. et al. Frontiers in Immunology 4, (2013).

Figure 3. Enhanced VCD of Human T cells. The viable cell number of human T cells in the presence of plasma HSA, commercial recombinant HSAs and deAlbumin[™] (0.5 mg/mL). Human T cells were treated with albumin in RPMI1640 supplemented with ITSE and lipid concentrate, IL-2 and activator (CD3, CD28, CD2) for 5 days.



Application: Animal-Free MSC CULTURE



Due to its self-renewal capacity, multilineage differentiation potential and immunomodulatory properties, mesenchymal stem cells have become candidates for cellular therapy of several diseases in humans.

However, properties of MSC still vary largely by the deriving tissue, the donor and the isolation technique applied (ref. 12). Since most MSCs progressively lose their multipotency and proliferation capacity in dishes, such inherent variation in cell properties have a great influence on the culture efficiency and quality of cultivated cells and hence the clinical outcome.

Therefore, it is crucial to use standardized culturing technique and culture media to yield consistent culture result along the project development from bench to bedside.

Today, it remains challenging to replace the undefined components such as fetal bovine serum (FBS) or human platelet lysate (hPL) with a mixture of chemical-defined components having comparable cell growth performance for MSC. Albumin is one frequently used component, when trying to substitue FBS and hPL (ref. 13). However, the influence of albumin's impurities, such as fatty acids, on MSC culture is also reported (ref. 14). By our albumin formulation technology, we could help you develope animal-free medium, which will consistently support high cell growth. Here, we show that deAlbumin[™] can boost MSC cell by 30-47%.

Additionally, our DA01 is an approved raw material for regenerative medicine by Japan PMDA with a "再生医療等製品材料適格 性確認書" certificate.



- 12. Estève, D. et al. Stem Cells International 2016, 1-8 (2016).
- 13. Schnitzler, A. C. et al. Biochemical Engineering Journal 108, 3–13 (2016).
- 14. Fillmore, N. et al. PLOS ONE 10, e0120257 (2015).

Figure 4. Human MSC grown with various albumin. The viable cell density of adipose MSC in the presence of plasma-derived HSA, commercial recombinant HSAs, and deAlbuminTM (0.5 mg/mL). After 2 passages in serum-containing medium (Day 0), hMSCs were treated with albumin in chemically defined medium (IMDM with the addition of TGF- β 1, FGF2, insulin, transferrin, mono-thioglycerol, L-Ascorbic acid, fibronectin and lipid concentrate) for additional 7 days before counting viable cell density.

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