



Recombinant Albumin

Its role in cryopreservation and cell recovery

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INTRODUCTION

Cryopreservation is widely used to generate large stocks or cell banks that can be stably stored for short and long periods ready to be recovered and used as required in both research and industrial applications. Demand is increasing for this step to be devoid of serum or any animal-derived components, especially when it precedes an animal-free biomanufacturing process.

Since one of the functions of albumin, the most abundant protein in serum, is as a carrier protein for many small molecules, it is a likely candidate to replace serum in cryopreservation. Recombinant albumin provides an animal-component-free (ACF) option without the risks and regulatory challenges associated with serum.

Here, we examine the ability for two different formats of ACF albumin products, CellPrime™ rAlbumin AF-G and CellPrime rAlbumin AF-S to replace serum in the cryopreservation of Chinese Hamster Ovary (CHO) and Sp2/0 cells, respectively.

Successful replacement of fetal bovine serum (FBS) with CellPrime recombinant human albumin, as shown in this study, allows scientists to develop defined serum-free ACF cryopreserving and recovery media.

MATERIALS AND METHODS

Cell lines:

CHO cell line expressing anti-IL-8 mAb; mouse myeloma cell line (Sp2/0) expressing anti-TGFβ-3 mAb.

Cryopreservation media:

Medium 1: FBS/DMSO

Medium 2: CellPrime rAlbumin AF-G, dimethyl sulfoxide (DMSO), chemically defined serum-free media (CD SFM) (CHO); CellPrime rAlbumin AF-S/DMSO/DMEM F12 (Sp2/0). Final concentration of CellPrime rAlbumins for both CHO and Sp2/0 is 23 mg/ml.

Medium 3: commercial cryoprotection medium

Culture conditions:

CHO: The cells were maintained in commercially available CD SFM, GlutaMax™, and Methotrexate (MTX). Cells were frozen at a density of 1×10^7 per vial in each cryopreservation media. After thawing, the cells were grown in CD SFM, in 50-ml minibioreactors. Cell performance was monitored over three days. Cell growth and viability were assessed daily.

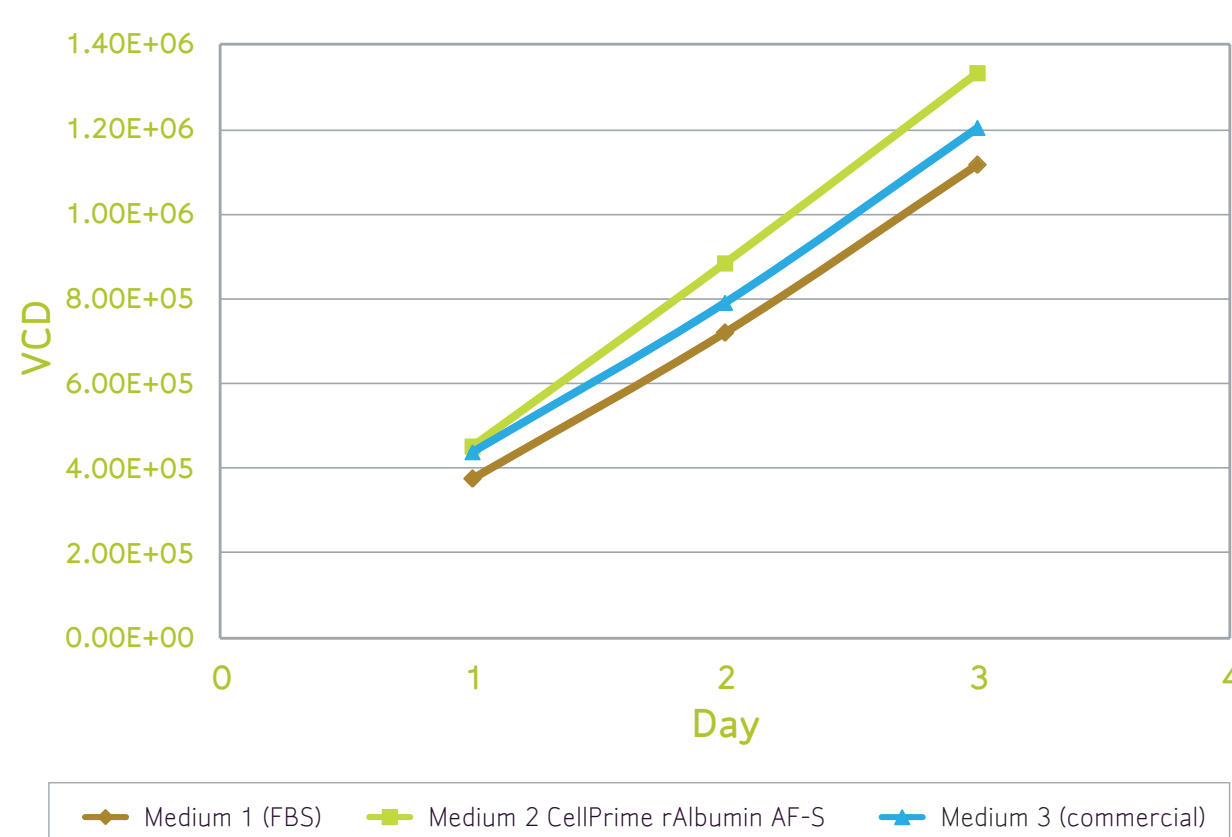
SP2/0: Cells were maintained in DMEM/F12, GlutaMax, insulin, transferrin, selenium (ITS), and 0.05% Probumin® Bovine Serum Albumin (BSA). The cells were frozen at a density of 5×10^6 per vial in each cryopreservation media. After thawing, the cells were grown in 25 ml of DMEM/F12, GlutaMax, ITS, and 0.05% Probumin BSA in T75 for three days. Cell growth and viability were assessed daily.

Performance parameters:

Viable cell density (VCD), % cell viability, and % mid-apoptosis were analyzed using the Guava® Viacount assay.

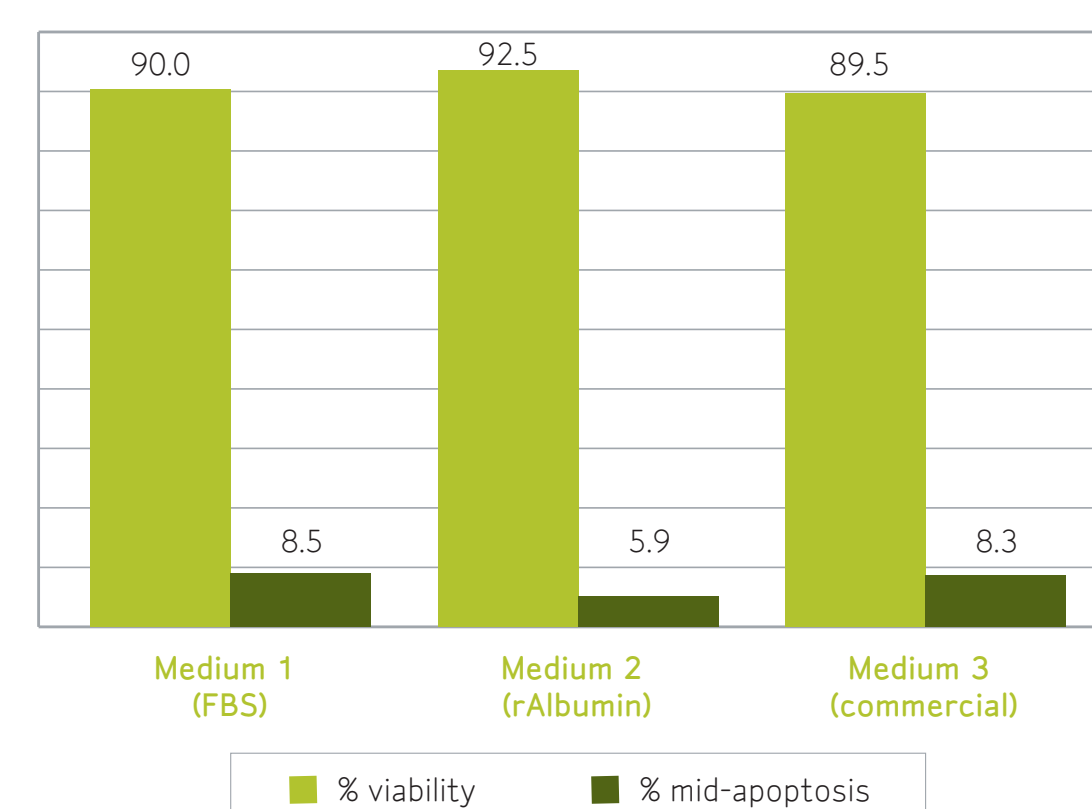
RESULTS: CHO Cells

Figure 1 Effect of cryopreservation media on growth (VCD) after thawing



- CHO cells frozen in three cryopreservation media containing FBS, CellPrime rAlbumin AF-G, or commercial medium demonstrated comparable growth over three days of recovery in CD SFM.
- CellPrime rAlbumin AF-G provided enhanced cell growth compared to FBS or a commercial medium.

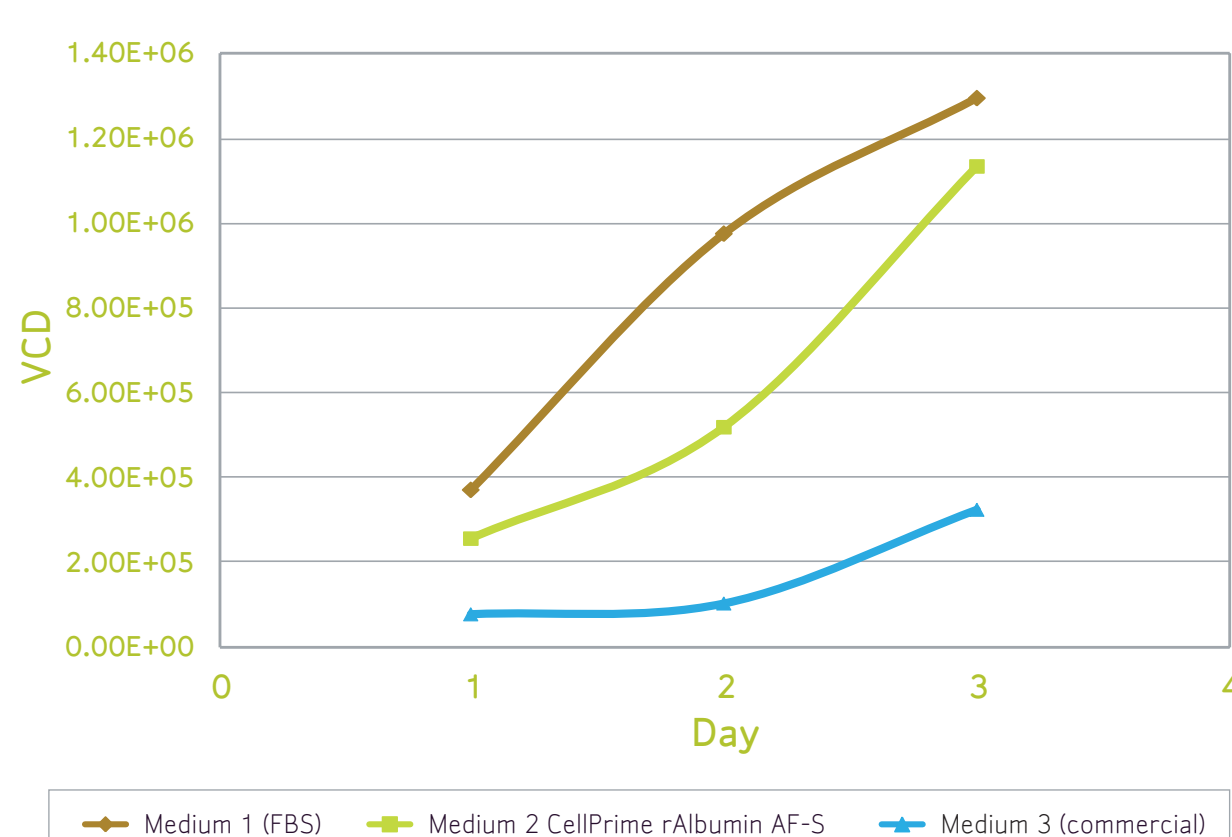
Figure 2 Effect of cryopreservation media on viability and mid-apoptosis after thawing



- After three days of growth, CHO cells frozen in media containing CellPrime rAlbumin AF-G demonstrated comparable viability to that of FBS and a commercial medium.
- The number of cells in mid-apoptosis was reduced for cells frozen in the presence of CellPrime rAlbumin AF-G.

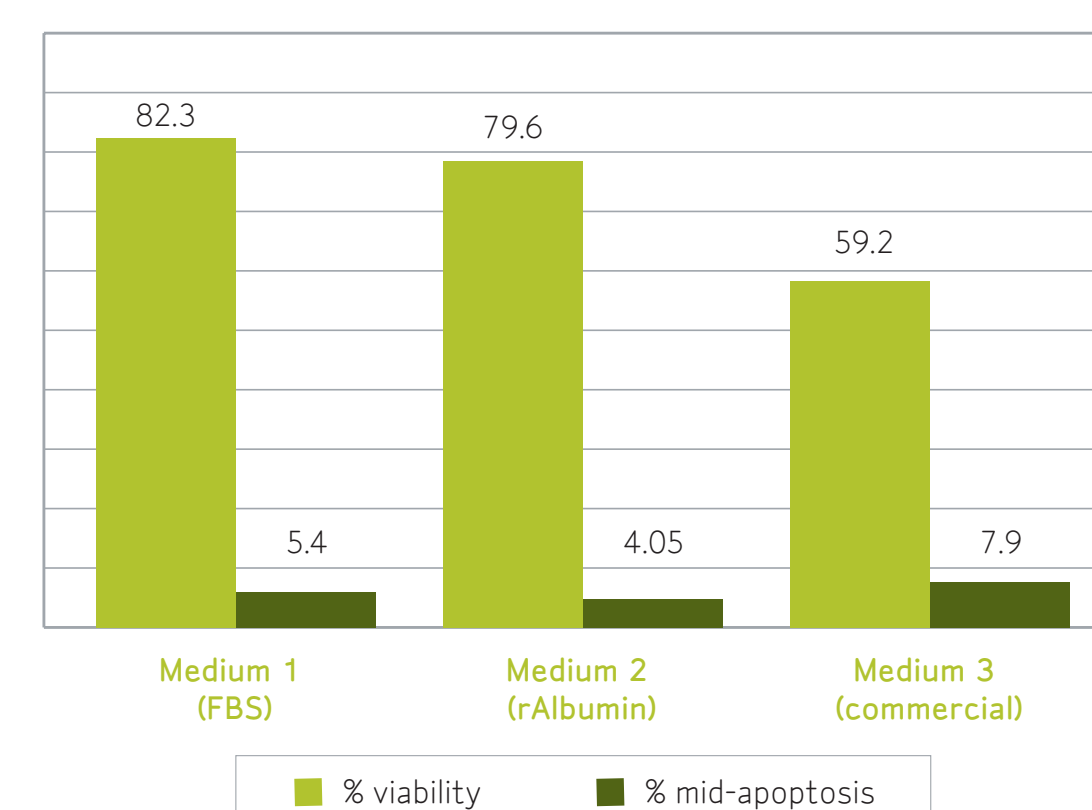
RESULTS: Sp2/0 Cells

Figure 3 Effect of cryopreservation media on growth (VCD) after thawing



- Sp2/0 cells frozen in three cryopreservation media containing FBS, CellPrime rAlbumin AF-S, or commercial medium. After three days of growth in CD SFM, Sp2/0 cells frozen in medium containing CellPrime rAlbumin AF-S demonstrated comparable growth to that of FBS and superior growth to a commercial medium.

Figure 4 Effect of cryopreservation media on viability and mid-apoptosis after thawing



- Like CHO cells, after three days of growth Sp2/0 cells frozen in medium containing CellPrime rAlbumin AF-S demonstrated similarly high viability and low % of cells in mid-apoptosis compared to FBS.
- Cells frozen in a commercial freezing medium did not recover with the same cell viability as those cells that were frozen in medium containing CellPrime rAlbumin AF-S.

CONCLUSION

- CellPrime rAlbumin AF-G and AF-S performed similarly to FBS and superior to a commercially available freezing medium in maintaining cell growth and viability
- They can be used to supplement both commercial complex media and basal media
- Cells frozen in the presence of CellPrime rAlbumin AF-G or AF-S maintained high cell viability and low levels of mid-apoptosis during recovery after thawing
- Either CellPrime rAlbumin AF-G and AF-S are suitable replacements for FBS and allows defined, ACF cryopreservation without compromising cell performance

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