TECHNICAL BULLETIN

Improve Cloning Efficiency with ClonaCell™-CHO ACF Supplement

Introduction

There has been exceptional growth in the production of biological drugs (or biologics) in cell culture systems over the past ten years, with the industry exceeding US$100 billion in sales in 2010.[1] Most of these biologics, including monoclonal antibodies, are produced in Chinese hamster ovary (CHO) cells, as these cells carry out the posttranslational modifications required for full biological function.[2-4]

Single-cell cloning is a necessary early step in the bioprocessing work flow. It is required for generating the cell line homogeneity that is crucial for consistent expression levels and quality attributes of the protein product.[5] There are several single-cell cloning techniques, including limiting dilution cloning (LDC), semi-solid cloning (SSC) and flow cytometry cloning.[6, 7] In LDC, a pool of transfected cells is diluted in order to seed culture wells with a single cell that is then expanded to a larger cell population. In SSC, individual cells are physically separated from each other in a semi-solid medium and each cell grows into a discrete, monoclonal colony. In flow cytometry cloning, a cell suspension is entrained in a narrow stream of liquid, such that cells flow in a single file with liquid separating each cell from the next. Cells are isolated using a vibrating mechanism that breaks the stream into single-cell-containing droplets that are deposited into individual cell culture wells.

Each of these techniques requires a cell culture medium that can support expansion of single cells. Supplementing the medium with fetal bovine serum (FBS) is the simplest method to cultivate and expand single cells with high efficiency. FBS, however, suffers from batch-to-batch variation and its use increases the risk of contamination from adventitious agents.[3, 8-10] Additionally, the use of FBS often requires re-adaption of cells from adherent to suspension growth after transition to serum-free conditions. Other approaches to support single-cell expansion without FBS include the use of conditioned media, co-culture with feeder cells or supplementation with hydrolysate. These methods also suffer from challenges related to batch-to-batch variation.[3, 11]

A large number of defined, animal component-free and protein-free liquid media for CHO cell culture are commercially available. When used alone, however, these media do not support efficient single-cell cloning. We have developed a defined, animal component-free 40X CHO supplement that only contains recombinant and synthetic components. In this paper, we demonstrate that this new CHO supplement dramatically increases the single-cell cloning efficiency of CHO cells when added to commercially available protein-free media.

Materials & Methods

Cell Culture

Suspension-adapted CHO (CHO-S) cells were maintained in ClonaCell™-CHO CD Liquid Medium (STEMCELL Technologies Inc.) supplemented with 6 mM L-glutamine (STEMCELL Technologies). Cell concentrations and viability were determined using a hematocytometer and trypan blue staining to exclude dead cells.

Single-Cell Cloning

CHO-S cells were diluted in Dulbecco's Modified Eagle Medium (DMEM) (STEMCELL Technologies) that was supplemented with 10% FBS, or in various protein-free media supplemented either with 6 mM L-glutamine and ClonaCell™-CHO ACF Supplement or with L-glutamine only. The following protein-free media were tested: ClonaCell™-CHO CD Liquid from STEMCELL Technologies, PowerCHO™ 2 from Lonza and CD CHO and CD OptiCHO™ from Invitrogen. Individual wells of 96-well plates were seeded with 200 µL of the respective test media at a cell density of approximately 1 cell/well. The plates were incubated in a humidified incubator at 37°C and 5% CO_2 for 14 days before screening to identify wells containing greater than 100 cells/well using an inverted microscope. Cloning efficiency was defined as the percentage of the total number of wells seeded that contained more than 100 cells/well after 14 days.

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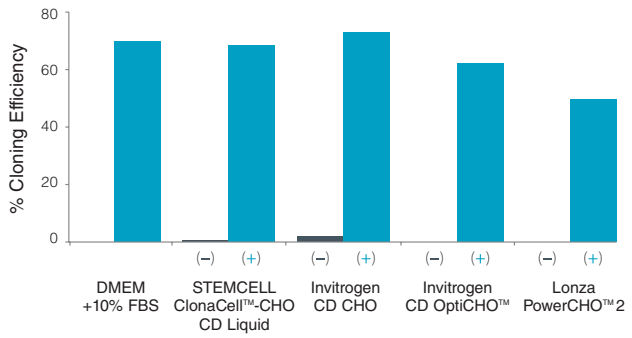
Results & Discussion

In order to characterize the ability of different media formulations to support single-cell cloning of CHO-S cells, we seeded CHO-S cells into wells of 96-well plates at an average concentration of 1 cell/well and examined each well for the presence of colony growth after 14 days of culture. Seventy percent of the wells cultured in DMEM supplemented with 10% FBS efficiently supported single-cell cloning as 70% of wells contained more than 100 cells after the 14-day culture period (Figure 1).

In contrast, very few of the wells seeded with CHO-S cells in any of the four chemically defined, protein-free media contained colonies, indicating that these media did not support efficient clonal expansion of CHO-S cells (Figure 1). When each of these media was supplemented with ClonaCell™-CHO ACF Supplement, however, the cloning efficiency dramatically increased, resulting in a similar percentage of growth-positive wells to that observed with serum-containing medium (Figure 1). Similar results were obtained using the Freestyle CHO-S cell line (data not shown).

In summary, our results show that ClonaCell™-CHO ACF Supplement can support high cloning efficiencies during limiting dilution cloning of CHO-S cells when combined with commercially available, chemically defined and protein-free media. This supplement should also be useful for other applications, such as supporting clonal cell growth after single-cell sorting, during semi-solid cloning in protein-free media and to support cell survival and growth after transfection.

FIGURE 1. Cloning efficiency of CHO-S cells in various cell culture media



The addition of ClonaCell™-CHO ACF Supplement to a variety of commercially available protein-free CHO cell culture media results in a dramatic increase in cloning efficiency for CHO-S cells.

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