Transitioning to Ultroser™ G

Techniques and Tips for Successful Cell Culture

Technical Note

This document provides an overview of the use of Sartorius Ultroser™ G, a serum substitute designed to replace animal-derived serum (fetal bovine serum (FBS) or fetal calf serum (FCS)) in mammalian cell culture. It covers the preparation, solubilization, and optimization of Ultroser™ G. The document also includes practical tips for general use, and detailed optimization processes to ensure optimal cell growth.

Introduction

Sartorius Ultroser™ G is a serum substitute that can successfully replace fetal bovine/calf serum (FBS, FCS) in cell culture medium. It has been developed for the *in vitro* culture of anchorage-dependent cells. It consists of a semi-defined composition ensuring batch-to-batch reproducibility with the added features of low protein content. It is supplied as a sterile, lyophilized powder which provides scientists with an easy-to-use and easy-to-store reagent (2-8 °C). Ultroser™ G serum substitute is designed to have a concentration five times higher than FBS (2 % of reconstituted Ultroser™ G serum substitute is equivalent to 10% FBS in the basal medium). This technical note will outline some of the recommended steps to consider when using this reagent. Further applications and details can be found on our website.

Tips for the routine use of Ultroser™ G

1. **Preparation and Solubilization.** The lyophilized powder should be solubilized in 20 mL sterile, distilled water as detailed in this document under sterile conditions. For greater sterility, the solution may be filtered through a sterilizing membrane of 0.2 µm pore diameter.
2. **Cell culture optimization.** After reconstitution, Ultroser™ G serum substitute has a biological activity 5 times greater than that of FBS. It should be used at concentrations between 0.5 % and 4 % (2.5 % to 20 % of FBS). A concentration of 2 % (10 % of FBS) is recommended but it is good practice to evaluate a range of concentrations to optimize as this may vary for each cell type. Consider growth dynamics and cell morphology during optimization.
3. **Cell culture media.** The culture medium may be the one generally used for culture with FBS (MEM, etc.). However, the use of an enriched medium such as HAM's F12, DMEM, IMDM, or RPMI is recommended.
4. **Culture Support.** The culture support is the same as that used with FBS. Ultroser™ G substitute contains adhesion factors. Therefore, it can be used for pre-incubation of the plastic dishes in the medium prior to adding the cells. However, plate coatings may be required for particular cell types, to improve adhesion and differentiation.

Optimization of cells for the addition of Ultroser™ G

Changing from FBS containing media to Ultroser™ G

- There are two suggested techniques to adapt cells to media containing Ultroser™ G.
- Technique one – Direct transfer of the cells in the medium supplemented with Ultroser™ G serum substitute. Some cells may display a level of sensitivity to this technique.
- Technique two – Using an adaptation period, or conditioning step, by progressively reducing the quantity of serum over several passages:
  - 1st passage: 50% medium supplemented with serum (MSS) + 50% medium supplemented with Ultroser™ G serum substitute (MSU) in the optimum concentration
  - 2nd passage: 25% MSS + 75% MSU
  - 3rd passage: 10% MSS + 90% MSU
  - 4th passage: 100% MSU

The second technique allows better adaptation of the cells to the new culture medium. In table 1 we outline some of the cell types we have evaluated that required some adaptation. Further examples can be found in the literature.

Cell type	Base media	Concentration of Ultroser™ G (%)	Transfer Technique
HT1080	Hams F-12K (Kaighn's)	2	Direct
A549	Hams F-12K (Kaighn's)	2 - 4	Conditioned
AU565	RPMI 1640	2	Conditioned
A172	DMEM	2	Direct
SKOV-3	DMEM	2	Direct
Fibroblasts (NHDF)	DMEM	0.5 - 1	Direct

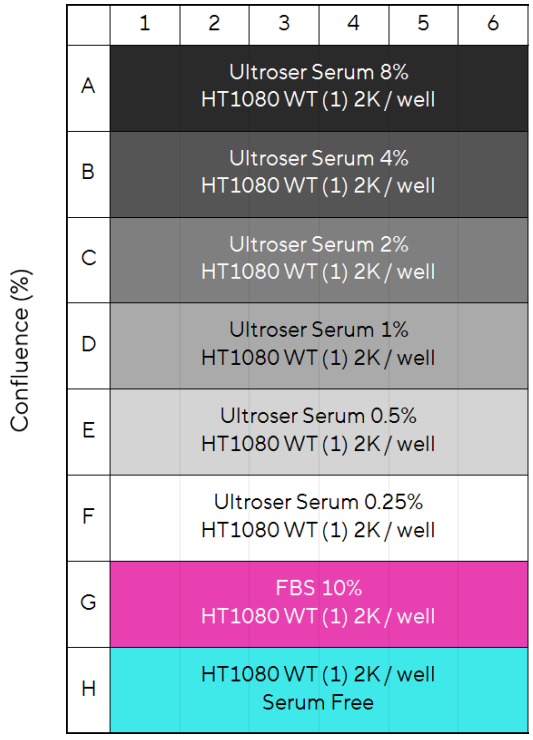
Table 1: Example cell line conditions, NHDF – normal human dermal fibroblasts

Optimization of Ultroser™ G concentration

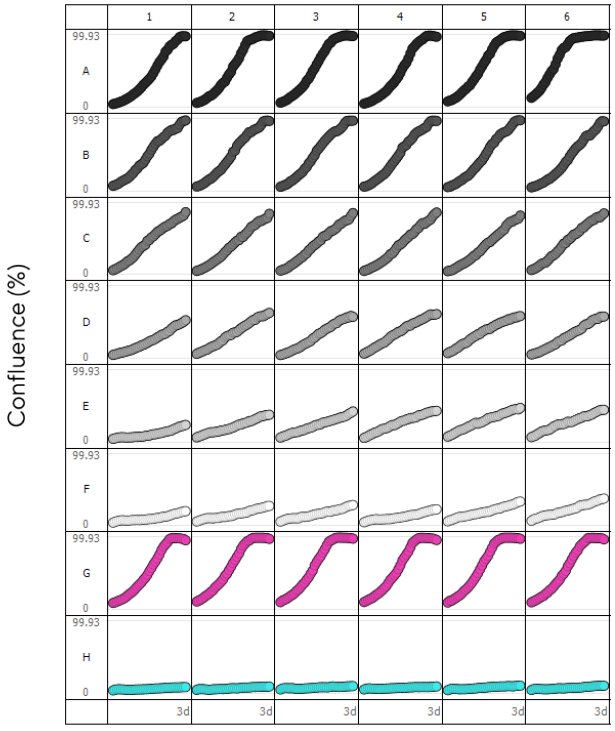
Generally, cells grown in 10 % FBS can be supported in a standard concentration of media supplemented with 2 % Ultroser™ G. For some cells it may be necessary to optimize the concentration used. The following steps demonstrate a suitable optimization process.

1. Set up media containing various concentrations of Ultroser™ G.
   - Suggest range from 0.5 % to 4 %
   - Higher concentrations may be detrimental to sensitive cell types.
2. Track cell growth to determine the optimal growth conditions for example using Incucyte® Live-Cell Analysis System (Figure 1).
   Note - The effects on cell growth are not always proportional to the concentration of Ultroser™ G serum substitute, nor in correlation with the results obtained with FBS in the same conditions of use.
3. Further optimization can be checked using a cell density gradient experiment (Figure 2).

A. Plate Map



B. Microplate Graph



C. Full Time Course

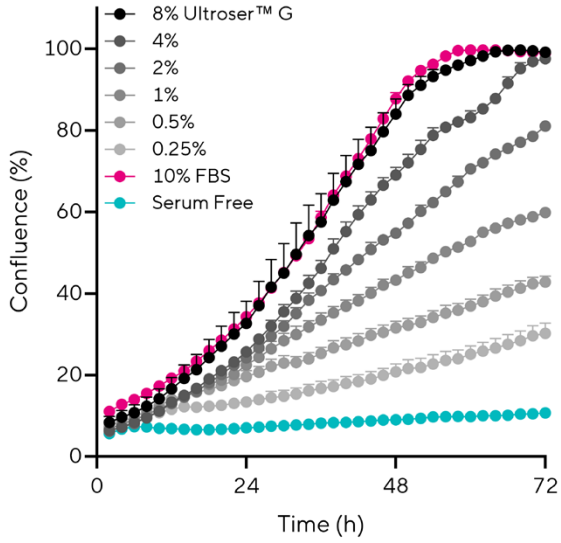
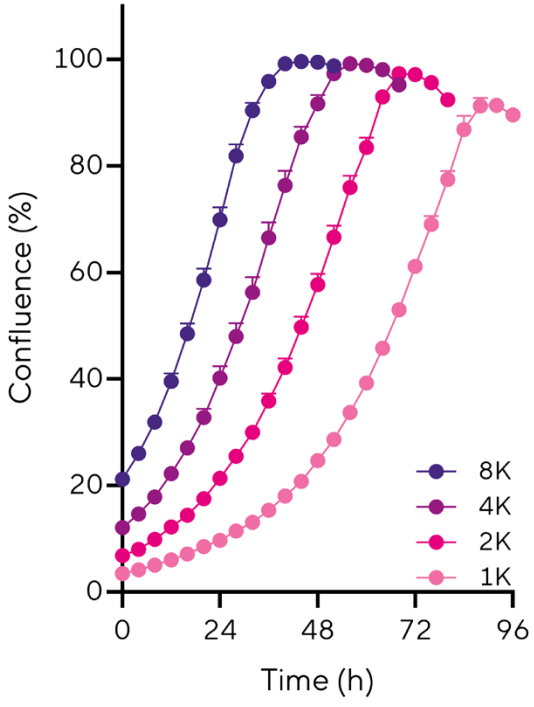


Figure 1: Example of growth optimization using HT1080 cells in combination with the Incucyte® Live-Cell Analysis System. (A) Cells were plated at 2K per well in various concentrations of media containing Ultroser™ G and (B) confluence of cells was quantified using AI confluence analysis over 4 days. (C) Time course graphs show growth in Ultroser™ G in comparison to media supplemented with 10 % FBS. Data shown as mean + SEM for 6 wells per condition.

A. Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	FBS 10% HT1080 WT (1) 8K / well								Ultroser Serum 2% HT1080 WT (1) 8K / well			
B												
C	FBS 10% HT1080 WT (1) 8K / well								Ultroser Serum 2% HT1080 WT (1) 4K / well			
D												
E	FBS 10% HT1080 WT (1) 8K / well								Ultroser Serum 2% HT1080 WT (1) 2K / well			
F												
G	FBS 10% HT1080 WT (1) 8K / well								Ultroser Serum 2% HT1080 WT (1) 1K / well			
H												

B. 10% FBS



C. 2% Ultroser™ G

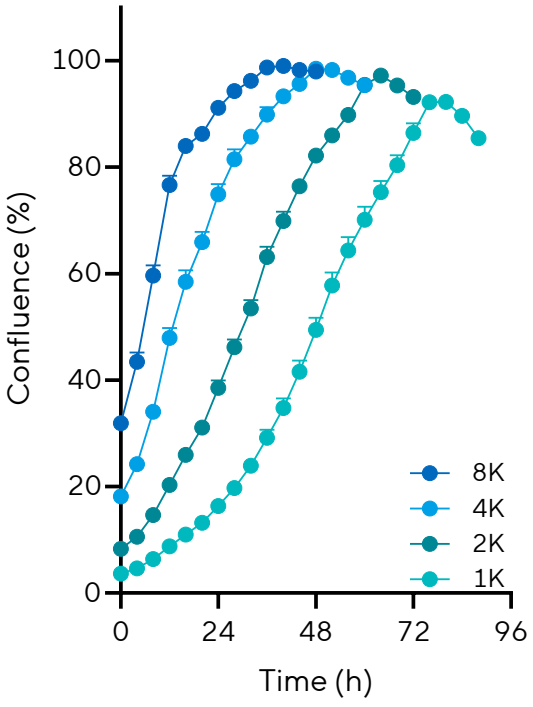


Figure 2: Example of further growth optimization using HT1080 cells in combination with the Incucyte® Live-Cell Analysis System. (A) Cells were plated at various densities in media containing either FBS (10 %) or Ultroser™ G (2 %) (B and C) Time course graphs of cell confluence, quantified using AI confluence analysis over 4 days for the two media types. Data shown as mean + SEM for 8 wells per conditions.

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