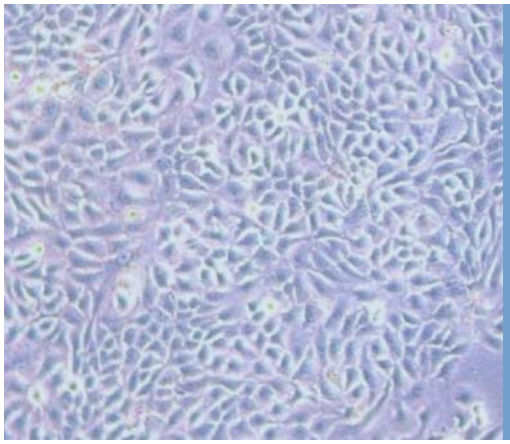

Protocol for using FastGro™, a fully defined, animal-component free cell culture supplement, to replace FBS

Performing cell culture without serum can be challenging. However, the rewards largely compensate for the efforts, and re-discovering the basics of cell culture quickly develops into a passion. The intention of this paper is to guide the user through a smooth transition to serum-free conditions while avoiding inadequate or wasted efforts.

Ideally, the transition to serum-free conditions should be carried out over several passages to gradually select cells that can grow under serum-free conditions. However, direct adaptation to serum-free environments are often successful, provided all crucial aspects are addressed properly. Regardless of the method used, key concerns include the growth state of the cellular inoculum, cell seeding density, sub-cultivation techniques, and biophysical attributes of the cell culture system.

MP Biomedicals' FastGro™ serum replacement is an effective FBS supplement and has been designed for use in the same manner as conventional cell culture sera.

IMPORTANT NOTES BEFORE USING CONCENTRATED FastGro™:

- FastGro is a cell culture medium additive replacing serum; it is not to be used as a final medium.
- FastGro must be added to a basal cell culture medium (e.g. IMDM, DMEM-F12 or any other basal medium of choice).
- FastGro does not contain growth factors such as cytokines, hormones, etc, and also lacks insulin*.

**The option to add insulin (or additional growth factors) is based on user requirements. If insulin is needed for cell growth and performance, we advise adding recombinant insulin at a concentration of 1.25 mg/L final cell culture medium.*

CONTENTS:

1 Preparing FastGro™

1.1 GENERAL PREPARATION OF SERUM-FREE CELL CULTURE MEDIUM

Gently shake the bottle of FastGro™ shortly before use.

Add FastGro™ to your preferred basal medium at the same concentration as for serum (e.g. 10%).

Do not add any antibiotics at this stage. Antibiotics, like many compounds, bind to the plasma proteins of serum, in particular to the albumin fraction. Thus, the same concentration of antibiotics will exhibit a much higher biological activity in serum- and albumin-free conditions, potentially impacting cell growth.

In instances where 'antibiotic-free culture' is deemed unworkable, the use of gentamycin is suggested at a concentration of 50 mg/L.

1.2 GENERAL ADAPTATION METHODS TO SERUM-FREE CONDITIONS

There are essentially two approaches to adapt cells to growth in a serum-free environment:

1.2.1 Direct Adaptation

This is carried out by a direct transfer of the cells from the serum-containing medium into the serum-free medium.

1.2.2 Sequential Adaptation or Weaning Method

Pass the cells from the original serum containing medium sequentially through phases where each step halves the serum-supplemented media, thus increasing the serum-free media to the following aproximate values:

Phase 1:
50% FastGro-supplemented medium/
50% Serum-supplemented medium

Phase 2:
75% FastGro-supplemented medium/
25% Serum-supplemented medium

Phase 3:
87.5% FastGro-supplemented medium/
12.5% Serum-supplemented medium

Phase 4:
93.75% FastGro-supplemented medium/
6.25% Serum-supplemented medium

Phase 5:
96.88% FastGro-supplemented medium/
3.12% Serum-supplemented medium

Phase 6:
98.44% FastGro-supplemented medium/
1.56% Serum-supplemented medium

Phase 7:
100% FastGro-supplemented medium

If reduced growth is observed, go back one step and continue after growth is reestablished.

Cell cultures may consist of cell lines (adherent or suspension growth) or primary cultures. Moreover, from a functional standpoint, cell types may be differentiated to various degrees or exhibit undifferentiated characteristics, as in the case of stem cell preparations. In each instance, the adaptation protocol must take into account the specific requirements of the cell type to guarantee the best chances for success.

2 Use of FastGro™

2.1 CELL LINES

The following protocols are valid for normal (diploid, limited lifespan), transformed or immortalized cell lines (with indefinite lifecycle).

2.1.1 Anchorage-dependent Cell Lines

Critical success factors:

- coating of the cell culture support for optimal cell attachment
- minimize action of trypsin
- choice of the antibiotic system

Experimental Steps:

A **Coat the cell culture surface with an adequate cell-attachment factor by using:**

- a commercial coating kit such as Pronectin™ F, MapTRIX™ or equivalent.
- a Fibronectin or Poly-L-Lysine coating, or a small quantity of FBS (e.g, 500 µL for a T25 flask) with overnight incubation at 37°C, followed by two washes with PBS or fresh medium.
- ready to use plastics that provide an improved attachment of adherent cells.

B **Dissociate the cell monolayer**

The use of standard trypsin preparations can become somewhat problematic in the absence of serum, which contains trypsin inhibitors. It is therefore important to minimize the proteolytic activity of residual trypsin in serum-free conditions to avoid irreversible damage to the cells. This is best achieved by utilizing trypsin inhibitors (e.g. from soybean) or by employing a non-mammalian dissociation reagent, such as Accutase™, which does not require inactivation or removal during passaging. Alternatively, an additional wash step of the cell pellet will remove the majority of the residual trypsin. However, this procedure employs an extra centrifugation step that can be damaging for some cell types.

Our preference is to omit trypsin and instead use Accutase™ or Detachin™ to dissociate the cell monolayer. These cell detachment solutions have been developed to meet the most demanding requirements for gentle and effective detachment of adherent cells. Cell membranes and surface epitopes will not be harmed during detachment, and the structural and functional quality of the surface proteins will remain intact.

C **Seed cells at 20,000 cells per cm^2 in complete medium as prepared under point 1**

It is important to observe a high seeding density during the first steps of the adaptation process. Cells normally secrete a host of factors into the culture medium that control cell attachment, growth and proliferation. However, these factors are absent in the fresh serum-free medium during the seeding step. A critical level of cell density is essential to induce immediate and sufficient production of these autocrine/paracrine factors.

D **Incubate and maintain the cell cultures at 37°C until they reach 80-90% confluency.**

During this period, change 75% of the medium every 2-3 days. Do not discard the spent medium. Instead, harvest the conditioned medium, sterile filter and put aside at 4°C for use in subsequent steps. If the cells appear stalled at any point, allow them more time to adapt to their new, serum-free environment.

E **When near confluency is reached, split the cells at a 1:2 or 1:3 ratio.**

For the second passage in FastGro™, a coating is not required, but use of conditioned medium is strongly suggested. This medium fraction contains autocrine factors that regulate attachment, spreading, growth and proliferation. Seed cells in a mixture consisting of 75% fresh medium and 25% conditioned medium, collected during the previous passage. Continue supplying cells with 75% fresh medium every 2-3 days and collect the conditioned medium as described in step **D** above.

F Repeat step **E** until the cells exhibit growth dynamics comparable to their former growth in serum-supplemented medium.
At this point, the cell line can be considered fully adapted. This may take up to a total of 4-6 passages.

G From this point on, antibiotics may be added to the culture medium.
We advise on the use of the large-spectrum antibiotic gentamicin; this antibiotic has a significantly reduced cytotoxicity as compared to the standard Penicillin/Streptomycin cocktails. The suggested concentration of gentamicin is 50 mg/L.

H Once adapted, the original split ratio (in serum-supplemented conditions) may be applied.

2.1.2 Anchorage-independent Cell Lines

The following protocol is adjusted for cell lines that already grow in suspension. For the adaptation of adherent cells to FastGro™ suspension growth, please see MP Bio's Application Notes for FastGro.

Critical success factor:
Choice of the antibiotic system

Experimental Steps:

A When cell densities of 3-5x10^6 cells/mL are reached (depending on the cell line) start switching to FastGro™ supplemented medium.
Harvest the cell suspension, take out a small aliquot for cell counting and centrifuge the entire suspension at 200 x g for 5 minutes.

B Perform a cell count according to your standard laboratory procedures.

C Resuspend the cell pellet in FastGro™ supplemented medium at a density of 10^6 cells/mL.
It is important to observe a high seeding density during the first steps of the adaptation process. Cells normally secrete a host of factors into the culture medium that control cell growth and proliferation. However, during the seeding step, these factors are absent in the fresh serum-free medium. A critical level of cell density is essential to induce immediate and sufficient production of these autocrine/paracrine factors.

D Incubate and maintain the cell cultures at 37°C until they reach a density of approximately 3-5x10^6 cells/mL.

E Split the suspension cultures at a 1:3 or 1:4 ratio by adding an appropriate volume of fresh medium (e.g. 25 mL of cell suspension and 75 mL FastGro™ supplemented medium, to be dispatched into 4 separate culture vessels)

F Repeat step **E** until the culture exhibits growth dynamics as originally observed in serum-supplemented medium.
At this point, the cell line can be considered fully adapted and may be split at the original ratios during serum-supplemented culture.

G Antibiotics may now be added to the culture medium.
We suggest the use of gentamicin at a concentration of 50 mg/liter; this antibiotic has a much lower cytotoxicity as compared to the standard Penicillin/Streptomycin cocktails.