FCS-free database: Practical tips to help you reduce/replace FCS use

Replacing FCS from your *in vitro* workflow may be challenging and may not always be possible - yet. Bear in mind though that the field is moving rapidly and that new cell culture supplements enter the market regularly. To help you on your way we share some practical tips!

Tip 1: Reduce FCS concentration – To start with, and if 100% replacement of FCS may not be possible, try to reduce the v/v %FCS in your cell culture medium. For many cell culture media 2.5-5% FCS is enough to maintain healthy, viable cells. They may proliferate a bit slower, but that may be solved by adaptation of your planning and cell culture regime. Consider that cutting down from 10 to 5% FCS in your cell culture media not only reduces FCS use, but also saves money from your precious research budget

Tip 2: Consider the use of 'low-serum media' – some media allow the use of lower concentrations of FCS (e.g. 0.5-2%) as compared to the standard 10% commonly used. Non-exhaustive list of low serum media: ITS from Defined Bioscience, "reduced serum" from Fisher Scientific, "reduced serum media" from VWR.

Tip 3: Replacing FCS – When you are determined to replace FCS from your *in vitro* workflow, be prepared for some testing. Obviously start by using the FCS-free database. If you cannot immediately find your specific cell type(s), search for similar cell lines/types (consider e.g. the germ layer from which your particular cell line/type originated). Also, consider universal FCS replacement options as a starting point that can be tweaked for your specific cell line/type. In addition, many media formulations have been optimized for specific cell types and these may also serve as a good starting point. Such media contain essential nutrients, growth factors, and supplements that may help replace FCS

Strategical points to consider:

1. **Adapt your cells to FCS-free growth conditions gradually**

If your cells are traditionally cultured in high-serum media, **gradually adapt them** to lower FCS concentrations. Reduce the serum concentration incrementally over several passages (e.g., from 10% to 7.5% to 5% and increase the amount of your serum-free alternative).

2. **Monitor cell viability and morphology (do not focus on proliferation)**

Do not focus on the potential to induce proliferation of your test reagents, but rather on the potential to support cell viability. You may lose some proliferative potential, but that is not necessarily a bad thing. You may find out that your new cell culture protocol reflects physiological conditions better than the old one.

3. **Use good cell culture practices**

While this may seem logical, it is often overseen. So please prewarm medium when initiating cultures, harvest cells gently and quickly while keeping exposure to proteolitic enzyme as short as possible. Also, make sure the proteolytic agents have been inactivated (serum-free please!). Keep cells suspension cold after harvesting to reduce the metabolic rate of the cells. And use enough volume and renew the cells culture frequently (e.g., every 2-3 days) to help maintain cell growth and remove metabolic waste, reducing the need for high-serum environments. Finally, check regularly that your cultures are not contaminated with mycoplasma.

4. **Some final tips**
   - **Consider changing your basic medium.** Sometimes high serum levels are necessary because the medium alone does not supply all of the nutrients required by the cells (e.g., EMEM, BME, DMEM). To counteract for those basic medium with missing micronutrients (e.g., proline addition in media for CHO cells line), switching to a more complex medium may help.
   - **Optimize seeding density**. Ensure you are using the optimal number of cells for each experiment. Too high densities may lead to excessive FCS consumption, while too low densities may require more serum for initial cell growth
   - **Optimize growth factors.** Use **recombinant growth factors** or cytokines to replace the components present in FCS that promote cell proliferation and survival. Supplement with albumin, insulin, transferrin, and lipids, which are critical components found in FCS and can be added to the culture medium to reduce reliance on serum.
   - **Attachment proteins (for adherent cell cultures)**. Beside the growth factors, the attachement proteins provided by the FCS might be necessary for adherent cell cultures. This can be conteracted by adding those attachement protein to the reduced serum medium or to coat with e.g. collagen. Another alternative is to use culture plates that are pre-coated with attachemnt proteins
   - **Test FCS-free cryopreservation methods.** While this may not lead to a great reduction of FCS use, it will make sure that your cells are not -temporarily- exposed to the proliferation-inducing stimuli that are present in FCS. Please check our Table for available cryopreservatives.
   - **Communicate your results to us.** Whether positive or negative, help your colleagues by sharing your results with us. We will try to incorporate these in the FCS-free database.

Further questions?

Contact us: 3RCU@uu.nl
3Rs Centre Utrecht
University Utrecht
University Corporate Offices
Bolognalaan 50 3584 CJ
Utrecht The Netherlands