The Cell Culture Dish

Ask the Expert
April 17th, 2015

Ask the Expert Session - Transient Protein Production in Mammalian Cells

This Ask the Expert will address questions in regards to transient protein production in mammalian cells. Mammalian transient expression allows for the rapid generation, purification, and characterization of milligram to gram quantities of secreted or intracellular recombinant proteins for therapeutic, functional, and structural studies.

Question:

How do you recommend verifying antibody expression in your transfection?

The Answer:

Most often researchers will use simple ELISAs or other immunoassay formats to check for antibody expression. These methods require relatively commonplace equipment and reagents, but can take longer to get to results. For serum and protein free media, if your antibody titers are high, you can actually get a relatively accurate look at antibody concentrations by running a simple Bradford assay since most of the protein in the cultures will be your antibody of interest. Lastly, there are high-end, label-free instruments such as the ForteBio Octet that can quantify antibody titers using Protein A sensors or similar.

Question:

We are using FBS in our culture, what steps would you recommend for removing FBS?

The Answer:

The first step is to identify a serum-free media formulation compatible with the cell line you are using. Typically serum is removed from cultures in a gradual, step-wise approach, replacing the serum containing media with the new serum free media with reduced levels of FBS in it. Wean the cells from the FBS by replacing with serum free media using FBS at about 75% of the original concentration and allow the cells to recover. Repeat with media containing 50% and 25% of the original FBS concentration until you are in completely serum-free conditions. If at any step cell health starts to be negatively impacted, go back to the previous concentration of serum and allow the cells to recover for a longer period of time before further reducing the FBS.

Question:

What cell density do you recommend before transfection in CHO cells?

The Answer:

Cells used for transient protein expression should be meticulously maintained from the time of thaw throughout their useful lifespan. Deleterious cell culture conditions can negatively impact not just your next transfection, but all transfections from that point forward. Cells should be well-characterized in terms of their growth rates and log phase growth range and should typically be split at mid log phase growth or slightly lower densities; do not let your cells overgrow and do not “over split” your cells by sub culturing the cells at low densities on multiple consecutive days. At the time of transfection, CHO cells, like most other cell types, should have high viability (95% or greater) and should be in low to mid log phase growth. Thus, for a CHO cell line with mid log phase at 4×10^6 viable cells/mL, transfecting cells when they reach a density of approximately 1-4×10^6 would be reasonable, with 2-3×10^6 likely being an optimal target density at the time of transfection.

Question:

I have read that pre-treating cells with DMSO can lead to higher yields, do you find this is true. Is there another yield raising method that does not involve DMSO?

The Answer:

The use of DMSO to enhance protein titers has been widely reported in the literature, however, we do not employ this method due to the toxic nature of DMSO as well as the undefined nature of its effects on cells. For certain cell lines, it may be advantageous to reduce the temperature of the cultures from 37C to 32C following transfection to shift the cell's usage of energy from replication to protein production. Other strategies include the use of compounds such as sodium butyrate to slow cell growth. As always, some of the best ways to improve protein yields are by ensuring that you have healthy cells and a fully optimized protocol encompassing the growth and maintenance of the cells, the DNA complexation reaction and the transfection of the cells themselves. Ensuring that your cells are happy and healthy goes a long way towards ensuring maximal protein titers.

Question:

If you want to express an intracellular protein could you still use a mammalian system? Which cell line?

The Answer:

Absolutely. While insect cells are still used quite a bit for expressing intracellular proteins, HEK293 cells also do a good job of expressing non-secreted proteins.

Question:

We have used transient production for a few projects, but are now looking at expanding our use. What kind of volumes could we reasonably expect if we scaled up and would you recommend HEK or CHO cells for high volumes? Also, if we optimized our system, what is the fastest timeline could we achieve?

The Answer:

Many researchers routinely use HEK293 cells at 1L scales in 3L shake flasks and at even larger volumes in 10L or 20L Wave bags or benchtop bioreactors. Certainly CHO cells can also be scaled up, as the vast majority of biotherapuetics are made in CHO cells at multi thousand-liter scales. Scaling up transient systems from mLs to liters is not trivial, and some cells will scale up better than others. When scaling up the dynamics in the shake flasks can vary considerably and one has to consider shake speeds, gas exchange, baffled vs. non-baffled flasks and temperature among other variables. At this time, I would recommend the use of HEK293 cells, as the yields from these cells tend to be considerably higher than CHO. Once again, the Expi293 system has been validated to generated consistent titers from multiwell plates all the way up to 10L Wave bags.

Question:

I am using a rather basic medium for my HEK293 cells and have seen mediocre transfection efficiency. I am wondering if I could get an increase in efficiency by simply using a richer media. If so, are there supplements you would recommend?

The Answer:

It is likely not simply an issue of using a "richer" media, but more so a better (i.e. possibly different) media that allows for more rapid growth of your cells so that the highest percentage of cells as possible are actively dividing at the time of transfection to promote DNA entry into the nucleus. There are many media developed specifically to support rapid cell growth and high density cultures and I would suggest screening some of the various 293 media available to find the one that works best for you rather than simply adding feeds or other supplements to your existing media. Gibco FreeStyle293 media and the newer Expi293 media are specifically designed to support high density HEK293 growth and transfection.

Question:

We are having some success in our HEK transfection but want to optimize the protocol to see if we can do better. What steps would you recommend and which do you think would have the most impact?

The Answer:

Unfortunately, this is a very difficult question to answer, as every step and each procedure plays a role in the success of your transfection. Always start be ensuring that your cells are as healthy as possible and have been cultured under controlled conditions, not letting the cells density get too high, or be too low, at the time of sub-culturing. Cell viability should be very high at the time of transfection. When generating transfection complexes, it is important to use a transfection reagent that has been shown to be compatible with your cells and to lead to high transfection efficiencies. Experiments should be performed to optimize the complexation media used as well as the amount of transfection reagent and plasmid DNA, the temperature of the reaction and the amount of time that complexes are allowed to form before addition to your cells. Also, be sure that highly charged inhibitory components (such as anti-clump) are not present in your transfection media. Thus, transient transfection should be viewed as more of a system in order to obtain optimal results rather than a collection of individual steps.

Question:

Which mammalian transient system is best suited for your needs? Hek293, CHO, others?

The Answer:

HEK293 is typically the cell line of choice for mammalian transient transfection due to their ease of transfection, high levels of protein expression and robust workflows. When proteins are being expressed for the purposes of therapeutic development, CHO cells tend to be the cell line of choice, as approximately 70% of licensed biotherapeutic proteins are produced in stably transfected CHO cells.