Getting Started with EZ-Flask for Hybridomas

Introduction

EZ-Flask (KDW0010) is a single compartment, 1 Litre flask bioreactor with a 200cm2 basal gas exchange membrane. After inoculation with hybridomas, or other suspension cells, the cells will gravitate to the base of the flask and settle above the gas permeable membrane. During the culture the cells remain directly above the gas interface and are therefore continuously oxygenated as they proliferate, with uninterrupted cell doubling, up to very high numbers. DO NOT AGITATE. After 3 to 4 weeks, 1 Litre of monoclonal antibody can be harvested without the need for passaging or other manipulations that require special care.

Recommended approaches

To initiate the culture, enough cells need to be introduced to create a growth-boosting environment. If your hybridoma grows easily in T-Flasks then only use Procedure 1 below. On the other hand, if your hybridoma is particularly difficult to grow, use Procedure 2.

Some hybridomas only produce antibody during exponential growth in EZ-Flask whereas others may continue to secrete antibody in the plateau phase or beyond. In the latter case waiting a full 30 days before harvesting will greatly optimize productivity.

It may be tempting to check cell numbers during the culture period but this is not recommended. Agitating the flask should be avoided.

Procedure 1 – "fill and forget"

Grow up around 25 to 50 million cells in T-Flask. **DAY 0 :** inoculate cells into 1 L of media (e.g. DMEM/10%FBS) in EZ Flask. Leave in incubator undisturbed. **DAY 20 to 30 :** Harvest 1 Litre of monoclonal antibody.

Procedure 2 – difficult hybridomas

DAY 0 inoculate 10 to 25 million cells into **250 ml** of media in EZ Flask. Adding some T-Flask conditioned media can help start the culture in EZ-Flask. **DAY 2 or 3** add **250 ml** to double the volume to 500ml. **DAY 7** add **500 ml** taking the total volume up to 1 L. **DAY 30** harvest 1 L of monoclonal antibody. (Principle - by starting with a low volume the cells can more readily condition the media which helps establish a robust culture.**)**

If Procedure 2 does not seem to work too well it could be that your hybridoma secretes an inhibitory cytokine. In this case try Procedure 1 and perhaps consider removing 500ml at day 15 and refeeding with the same volume of fresh media.

Re-feeding the culture with fresh media

Re-feeding the flask with fresh media is possible and in this way the culture can be maintained for longer periods if desired, eg take out 500ml at day 20 - 25 and replace with fresh.
Note - A built-in pipette-stop facilitates pipetting media from the flask without disturbing the cells.

Counting cells

To count the cells :

- First remove media using a pipette, leaving around 200ml.
- Place the cap back on the flask and resuspend the cells by planar agitation (not shaking).
- Once cells are resuspended, tilt the EZ flask so that the media and cells are near the EZ flask opening.
- Remove a sample of cells from the remaining media volume.

Glucose measurements – note that the consumption of glucose by hybridomas in EZ-Flask is proportional to the number of viable cells. It is not required to track glucose in EZ Flask but if you would find it interesting and useful to do so, an easy-to-use glucose meter that is calibrated for cell culture media, is available from KDBIO.