Getting Started with EZ-Flask for Hybridomas

Introduction

EZ Flask is a unique and very simple-to-use hybridoma culture flask. It has a gas exchange membrane at its base to permit efficient and continuous oxygenation over a long period of incubation without the need for cell passaging. The base membrane surface area is 200cm².

Some clones produce more antibody during the exponential growth phase and other clones may produce more antibody after the culture has reached its growth plateau when the cells are stressed. This means that when starting with a new clone some process optimisation can be useful to achieve best results. See below for "Optimizing with G-Rex 6-Well Plate".

Below are two commonly used procedures. In both cases, for hybridomas we recommend a minimum of 50 million cells to be inoculated in order to achieve a sufficient membrane surface density to initiate the culture. With optimisation, you may find it possible to reduce the number of cells for inoculation.

IMPORTANT: when inoculating EZ Flask/G-Rex please bear in mind that the most meaningful parameter of reference is: *cells per cm² of base membrane*.

Example procedures

Example 1 – favours late-producer clones

Grow 50 to 100 million cells in T-Flask(s).
DAY 0 : inoculate 1 L of media (e.g. DMEM/10%FBS) in EZ Flask. Leave in incubator undisturbed. **DAY 30 :** Harvest 1 Litre of monoclonal antibody (or wait until cell viability down to 10% then harvest).

Example 2 – for faster culture

Grow 50 to 100 million cells in T-flask(s).
DAY 0 inoculate cells into **250ml** of media (e.g. DMEM/10%FBS).
DAY 2 or 3 add **250ml** to double the volume to 500ml.
DAY 7 add 500ml to make the total volume up to 1 Litre.
DAY 14 – 16 harvest 1 Litre of monoclonal antibody.

Optimizing with the G-Rex 6-Well Plate

The G-Rex Plate provides a convenient way to experiment with different conditions on a small scale to better understand how to achieve best productivity with the EZ-Flask. It provides an ideal method to test, say, 3 different hybridomas with the suggested procedures to determine whether the clone is an "early producer" or "late producer". A single hybridoma can also be tested with different media or seeding densities, for example. Each well of the G-Rex Plate can be used as a scaled-down version of the EZ-Flask.
Individual well specifications: 10cm² gas permeable membrane with a 35mL media capacity.